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ATMOSPHERIC HUMIDITY AND TEMPERATURE IN RELATION TO THE WATER SYSTEM OF PLANTS AND SOILS[1]

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(WITH FOUR FIGURES)

Introduction

From the very beginnings of plant physiology as an experimental science, the water relations of plants have received much attention. During two thousand years before VAN HELMONT's classic experiment with the willow shoot, Grecian thought had dominated the field of natural history. Plants were believed to receive from the earth, through their lacteal roots, a perfect pabulum from which their entire bodies could be constructed, with nothing needed for growth lacking, and, since there were no obvious excretions, nothing ever left over as waste. The earth was likened to a gigantic stomach within whose recesses the food of plants was digested and elaborated into a form corresponding exactly to the plant's needs. A single building substance was believed to be taken in through the roots, distributed through fibrous vessels to all parts of the plant body, and used in the complete construction and nourishment of all of its organs.

The first experiment in plant physiology is usually ascribed to VAN HELMONT, who was one of the most distinguished iatro-chemists of his period. When VAN HELMONT in 1610 concluded from his experiment that the 164 pounds, three ounces of dry weight of willow wood, bark, roots, and leaves had been constructed entirely from the rain or distilled water which he had supplied it, his conclusion was typically Grecian. He merely had proved to his own satisfaction that water alone was that perfect pabulum of plants. The fact that 2 ounces of earth was lost in some manner during the 5 years did not disturb him. If he thought at all about this loss of earth, he must have felt as we moderns are wont to do, that a loss of only slightly more than

[1] Fourth STEPHEN HALES address, read before the American Society of Plant Physiologists at St. Louis, December 31, 1935.

0.06 per cent. of the earth was insignificant in view of the fact that 164 pounds of dry matter had been formed.

This experiment was not left long unchallenged, however; the work of GLAUBER on salt petre in 1656, and the beautiful experiments of WOODWARD (**28**) on spearmints in 1699 fully confuted VAN HELMONT'S views. These spearmints were grown in water from different sources, and were found to flourish somewhat in proportion to the amount of earthy contamination which occurred in the water. Insisting that it is the earthy material itself that serves as the pabulum of plants, JETHRO TULL (**26**) remarks concerning the early "water philosophers," that they "were deceived, in not observing that water has always in its intervals a charge of earth, from which no art can free it." That VAN HELMONT should have drawn unwarranted conclusions from a well-planned, well-executed experiment is not to his discredit. It merely indicates the difficulty of original work at this early period, and it may also stand as a warning to all of us who in our own age share with him the frailty of being human and of occasionally making errors in the interpretation of our results.

The most important early investigator of water relations of plants was STEPHEN HALES (**4**), to whom we annually pay our tribute of respect and honor for his contributions in that field. His interesting quantitative measurements were many years in advance of the period in which he lived. They were made long before osmotic phenomena had been discovered and rationalized in terms of plant behavior; before either hydrogen or oxygen had been isolated as elements; more than 50 years before CAVENDISH announced to the world the synthesis of water from its two constituent elements, thus proving its constitution; more than a hundred years before the establishment of the cell generalization by SCHLEIDEN and SCHWANN; more than a hundred years before the description and recognition of protoplasm as the fundamental living substance of living plants and animals. Many of HALES'S experiments are worthy of attention today. He measured the amount of water evaporated from the leaves of plants, measured the areas of root systems and total leaf areas of plants, from which he calculated the thickness of the layer of water absorbed by the root system in a 12-hour period, and the thickness of the layer of water given off by the leaves during the same time. From these studies he estimated in a crude way the rate of sap rise. His approach to this problem was the same as that recently used by CRAFTS (**2**) in his early attempts to account for phloem transfer of organic foods in cell walls. HALES compared the transpiration rate during the day with the rate at night, using methods that would make beautiful laboratory demonstrations for our classes today. He compared water loss from leafy and leafless twigs, and from deciduous and evergreen trees. He calculated the amount of water given off by an acre of hop vines in terms of a layer of water over the entire area. He studied root pressure (and also

blood pressure of animals) and made the first use of mercury manometers on decapitated branches of grape vines to measure their root pressures. He demonstrated the suction power of evaporation not only by means of severed branches, but by means of the roots of living trees. He captured and condensed the vapor exuded by leaves to determine whether it differed in any way from ordinary water. He investigated the possibility of translocation of odoriferous solutes through the stems to leaves and fruits. He proved that sap rise continues during winter. He measured changes in soil humidity and temperature through the seasons, and attempted to estimate the value of dew in the water economy of plants. He compared rainfall quantitatively with transpiration. He attempted to measure the imbibitional swelling of dry peas, and carried on a series of ringing experiments designed to show whether or not there is a circulation of sap in plants. He concluded that there is no real circulation of the sap, although he admits that sap can pass down the stems under certain conditions. He also made use of an instrument which he attached to grape vines to measure diametral contraction and expansion of their stems with water loss and water gain respectively. With a sensitivity of 0.01 inch, he was able to observe such expansion of vine stems during rains. These observations were the forerunner of modern dendrograph records.

It is not difficult to find defects in his work, inept reasoning, faulty interpretation, and lack of understanding of the plant body; but these contributions, considered with reference to the general state of knowledge in the early 18th century, form an unique and remarkable foundation for the great advancement which has been made since his time. The expanding literature on water relations attests year after year the perennial interest in these problems. The reason for this interest lies in the realization that there is no more fundamental relation than that of the active organism to its internal and external water supply. During the last 60 years we have seen the problems of imbibition and osmotic behavior analyzed in considerable detail as they relate to cellular physiology and to the life of the plant as a whole. Methods of measuring these relations with a fair degree of accuracy have been developed. We have seen also the gradual development of a consistent hypothesis to account for the ascent of sap in plants. The cohesion theory is at once mentally satisfying and challenging as a working hypothesis. At the same time, it must be considered only an hypothesis, and must be subjected to testing in every possible manner. We are also coming to see and to understand more fully the basic importance of relative humidity and temperature of the atmosphere in relation to the water system of plants and soils. It is the purpose of this discussion to focus attention upon the fundamental causes of changes in these water systems as these changes relate to the physiology of the organism.

Forces of evaporation

It is now generally recognized that evaporation of water from the exposed aerial portion of plants is an unavoidable consequence of the differences in magnitude between the vapor pressure of the moist biocolloids of the plant body and of the vapor pressure of the surrounding atmosphere. The molecules of water held on the surfaces of these biocolloids are kinetically active, and some of them move with sufficient momentum to break loose from the surface attractions and diffuse into the atmosphere. How powerful are these movements? What force must be applied to the molecules to inhibit their escape, or to bring about the state known as dynamic equilibrium? The magnitude of the force required to establish such water equilibrium depends upon the condition of the atmosphere itself. In a nearly saturated atmosphere, the force required is very small, while in an extremely dry atmosphere the force required is very large. An attempt will be made to clarify this problem.

The tendency of water to escape from the colloidal cellular substrate into the environment may be reduced in several ways: (1) Lowering the temperature of the leaf decreases the kinetic motion of the water molecules, so that their momentum does not so frequently tear them loose; moreover, it reduces the water vapor tension of the internal atmosphere of the leaf, thus decreasing the vapor pressure gradient which carries the vapor away from the colloidal surfaces by diffusion. (2) Increasing the relative humidity of the air decreases the outward diffusion of water also, by reducing the vapor pressure difference or gradient of diffusion. (3) Increasing the internal forces, either of the water itself (as by increase of hydrated solute constituents), or of the surface attractions of the biocolloids for water, whether by chemical modification of the colloid or by mere drying, causes greater resistance to the kinetic action of the water molecules.

As has been shown in a recent paper (7), it is possible to calculate the force which must be applied to the water molecules to balance their escaping tendency from the colloidal surfaces, or to bring them into dynamic equilibrium with their environment. This force varies directly with the water vapor deficit[2] and temperature of the surrounding air. For every combination of these two factors there is a corresponding colloidal condition in which the forces of water movement are in equilibrium. The colloidal state of dryness at this point is a matter of empirical test; one must determine it by

[2] In table I the humidity relations are expressed in the usual manner, as percentage of saturation; it should be noted, however, that in the equation used for calculations, P_v represents saturation (100 per cent. relative humidity), and P'_v is the actually observed relative humidity, determined empirically for some definite time and place, *e.g.*, 80 per cent. relative humidity. $P_v - P'_v$ is thus an expression of saturation deficit of the atmosphere, and in a calculation for this particular degree of saturation deficit (20 per cent.) becomes log 100 – log 80, or 2.00000 – 1.90309 = 0.09691.

measurement; but the *forces* at equilibrium are calculable, and do not need to be measured.

The thermodynamic equation used in making such calculations was first used more than 50 years ago, but it has not found use in connection with the interpretation of water relations of plants and soils until recently. The equation is easily derived, and in the integrated form has the following expression: $\triangle P_1 = \frac{RT}{V_1} \cdot \ln \frac{P_v}{P'_v}$, which for purposes of calculation may be expressed in the Briggsian system as $2.3026 \cdot \frac{RT}{V_1} \cdot \log \frac{P_v}{P'_v}$. The effectiveness of the environment in providing a partial water vacuum into which evaporation from living plants may occur is indicated in table I. The forces which would exist on the biocolloids and in the atmosphere at dynamic equilibrium have been calculated for sixteen different degrees of relative humidity (saturation deficit), and six different temperature values. These humidities and temperatures have been chosen to cover approximately the entire range of conditions presented by the natural environment of our land flora. Relative humidity seldom goes below 10 per cent., except possibly in the driest desert regions of the earth; and the biokinetic range of temperature for ordinary land plants may be considered as lying between 0° C. and 50° C. Seeds and spores in the dry state can withstand the lowest temperatures that man can produce, within a degree or so of absolute zero, without loss of life (**9**); but the temperature for active growth usually lies between the limits stated. The values presented in table I have been subjected to a minor correction[3] for the changing values of the molal volume of water with changing temperatures. For purposes of theoretical discussion the figures are, therefore, sufficiently accurate. They give an illuminating picture of the water conditions of the atmosphere throughout the range of relative humidity and temperatures to which living organisms are normally exposed.

In earlier papers (**17, 18, 19, 21**) the conclusion had been reached that dry seeds, when in dynamic equilibrium with their environment, were holding their hygroscopic water with a force of from 950 to 1350 atmospheres. From table I it may be seen at a glance that the corresponding humidities of the atmosphere which would generate forces with such values, would be in the neighborhood of 40 to 50 per cent. relative humidity. This is not an unusual value for the humidity of laboratory air, which is usually much drier than the outside atmosphere.

[3] The values of V_1 used in the calculations are as follows:

0° C., 18×1.00013	30° C., 18×1.00436
10° C., 18×1.00027	40° C., 18×1.00782
20° C., 18×1.00177	50° C., 18×1.01207

These values were obtained from HODGMAN'S Handbook of chemistry and physics, 19th ed., p. 1115, 1934.

TABLE I

TENSIONAL FORCES REQUIRED TO STOP EVAPORATION (DYNAMIC EQUILIBRIUM) EXPRESSED IN ATMOSPHERES FOR VARIOUS RELATIVE HUMIDITIES AND TEMPERATURES THROUGHOUT THE BIOKINETIC RANGE

RELATIVE HUMIDITY	TEMPERATURE RANGE, °C.					
	0°	10°	20°	30°	40°	50°
%	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*
10	2865.4	2969.9	3070.3	3166.9	3260.1	3350.2
20	2002.8	2075.9	2146.0	2213.6	2278.7	2341.7
30	1498.25	1552.9	1605.4	1655.9	1704.7	1751.7
40	1140.25	1181.9	1221.8	1260.2	1297.3	1332.6
50	862.6	894.0	924.2	953.3	981.4	1008.5
60	635.7	658.9	681.1	702.6	723.26	743.2
70	443.8	460.1	475.6	485.0	505.0	518.9
80	277.7	287.8	297.5	306.9	315.9	324.7
90	131.1	135.9	140.5	144.9	149.2	153.3
95	63.8	66.2	68.4	70.56	72.6	74.6
96	50.8	52.7	54.4	56.1	57.8	59.4
97	37.9	39.3	40.6	41.9	43.1	44.3
98	25.1	26.05	26.93	27.8	28.6	29.4
99	12.49	12.95	13.39	13.8	14.2	14.6
99.5	6.25	6.47	6.69	6.9	7.1	7.3
99.9	1.23	1.28	1.32	1.36	1.4	1.44

The data of table I reveal that vapor pressure deficit is a much more important factor in determining the final value of the equilibrium condition than is temperature. The influence of a rise in temperature in creating greater equilibrium forces is seen by reading the lines of figures from left to right across the table. At a relative humidity of 95 per cent. (deficit 5 per cent.), a 10° rise in temperature increases the forces of dynamic equilibrium by the small amount of 2 to 2.4 atmospheres; and at 99.9 per cent. relative humidity, even a 50° rise in temperature raises the force at dynamic equilibrium by only 0.21 atm. At low humidities, however, the temperature effect is very much greater, a difference of 10° at 10 per cent. relative humidity increasing the forces at equilibrium by 90 to 100 atmospheres.

It is easier to visualize the fundamental difference in the magnitude of the effects of humidity (deficit) and temperature from graphs of the data in table I. In figure 1 the data are plotted to show changes in the equilibrium forces with temperature change. The increasing steepness of slope of the lines toward the top of the graph shows the increasing effects of temperature as the humidity decreases. These sloping lines, if projected to the left, will converge at absolute zero, approximately. The change in slope is just what would be obtained if the plotted distance from −273° C. to 50° C. were to be used as the radius of a circle which rotated through the range of naturally occurring humidities.

Still more striking are the curves of figure 2, which show the rapid in-

crease in the equilibrium forces with decreasing humidity (increasing vapor pressure deficit). The lower curve in this graph represents the changes with decreasing relative humidity when the temperature is maintained at zero; and the upper curve is plotted for the same changes in humidity with a maintained temperature of 50° C. The curves for 10°, 20°, 30°, and 40° C. would lie between those shown in the graph, and would pass, respectively, through the four points indicated between the curves shown. The tempera-

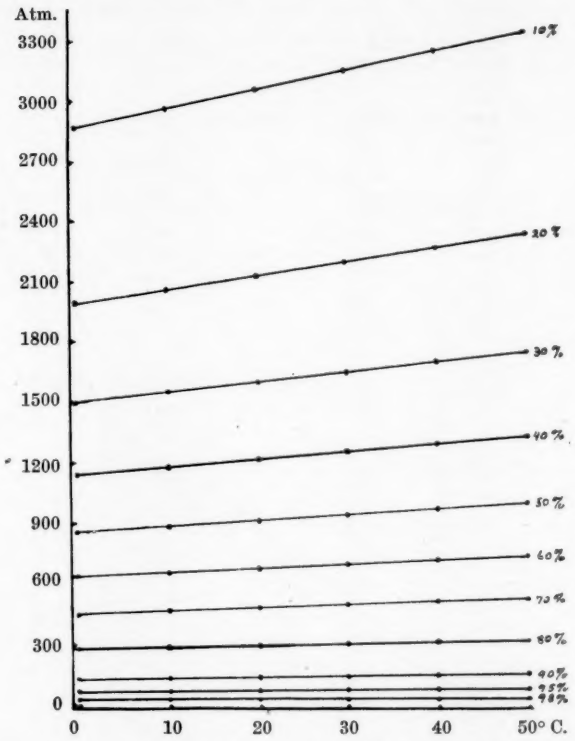

FIG. 1. Increases in the equilibrium forces with temperature increases.

ture influence is indicated by the vertical distance between homologous points on the two curves. The vertical distance between the curves becomes much greater with low humidities than with high. It should be clear from this graphic representation of the data that, of the two factors under consideration, the vapor pressure deficit is much more important than temperature in determining the character of the atmosphere with reference to water equilibrium conditions.

Wind flow is a factor in determining the *rate* at which the system will approach equilibrium, mainly because it sweeps moisture away from the leaf as rapidly as it diffuses through the open stomata. This brings the full

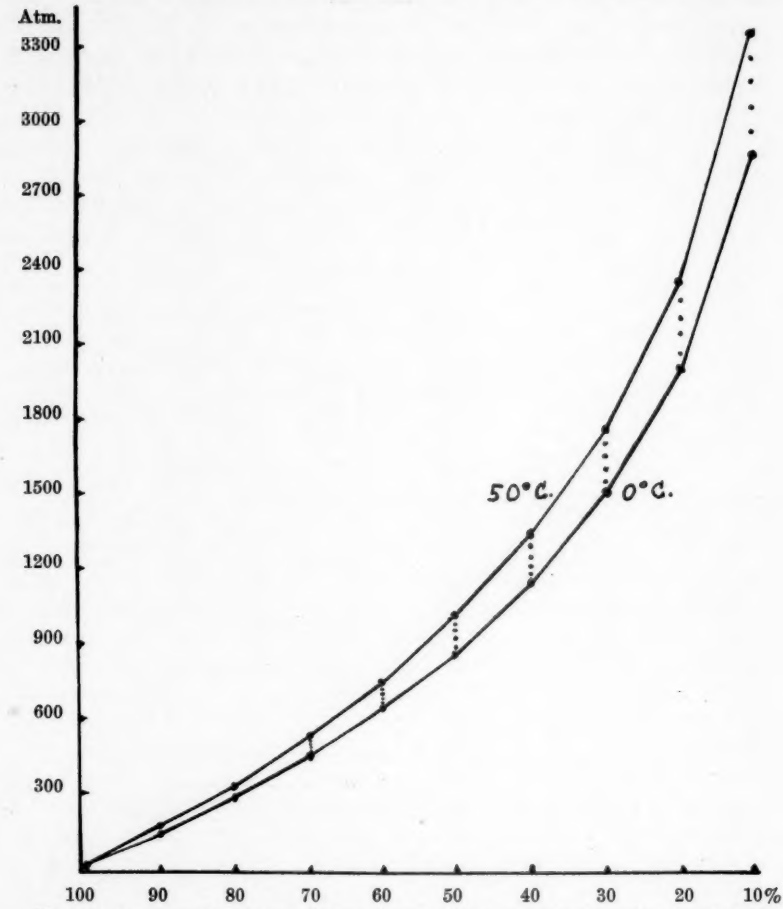

FIG. 2. Increases in the equilibrium forces with increasing atmospheric saturation deficit.

effects of atmospheric aridity to the surface of the guard cells, and into contact with the cutinized surfaces of the epidermal cells. MARTIN and CLEMENTS (**10**) have just shown that air flow of 1 mile per hour, approximately 1.5 ft. per second, causes a 30 per cent. increase in the transpiration of young sunflowers. Increasing the air flow to 16 miles per hour did not

suffice to double this effect. These results show that when the air flows rapidly enough to remove the diffusing water, additional air flow has only slight effect until stomatal closure is induced, which MARTIN and CLEMENTS found to occur at an air flow of about 5 miles per hour in the case of *Helianthus annuus*.

The atmosphere constantly fluctuates in relative humidity and temperature, with the result that the conditions for water equilibrium between the plant and its environment are constantly changing. Sometimes the changes are very large in a very brief period. To illustrate the changing conditions in concrete fashion, the U. S. Weather Bureau data from Washington, D. C., for 10 days in June, 1932, have been used as a basis for calculating the equilibrium water relations in atmospheres. Table II shows the combinations

TABLE II

DIURNAL FLUCTUATIONS OF EQUILIBRIUM FORCES AT WASHINGTON, D. C., JUNE, 1932

DATE		RELATIVE HUMIDITY	TEMPERATURE	EQUILIBRIUM
		%	°C.	atm.
6/16/1932	8:00 A.M.	94	21	83
	Noon	78	24	336
	8:00 P.M.	90	21	141
6/17	8:00 A.M.	84	21	233
	Noon	56	27	790
	8:00 P.M.	77	25	354
6/18	8:00 A.M.	81	19	280
	Noon	80	18	296
	8:00 P.M.	86	17	199
6/19	8:00 A.M.	94	17	82
	Noon	81	19	280
	8:00 P.M.	85	19	216
6/20	8:00 A.M.	92	18	110
	Noon	76	21	367
	8:00 P.M.	80	22	299
6/21	8:00 A.M.	78	22	333
	Noon	68	27	526
	8:00 P.M.	74	27	410
6/22	8:00 A.M.	66	26	565
	Noon	50	30	953
	8:00 P.M.	53	30	873
6/23	8:00 A.M.	53	26	862
	Noon	45	22	1072
	8:00 P.M.	39	25	1275
6/24	8:00 A.M.	38	17	1278
	Noon	24	21	1909
	8:00 P.M.	29	25	1677
6/25	8:00 A.M.	67	21	536
	Noon	32	29	1562
	8:00 P.M.	40	29	1256

of relative humidity and temperature at 8:00 A.M., noon, and 8:00 P.M. from June 16 to June 25 inclusive, and the internal tensions which would exist in the plant at dynamic water equilibrium with the air under these conditions are shown in the last column of table II. The fluctuations are depicted in figure 3, which shows that equilibrium conditions are usually

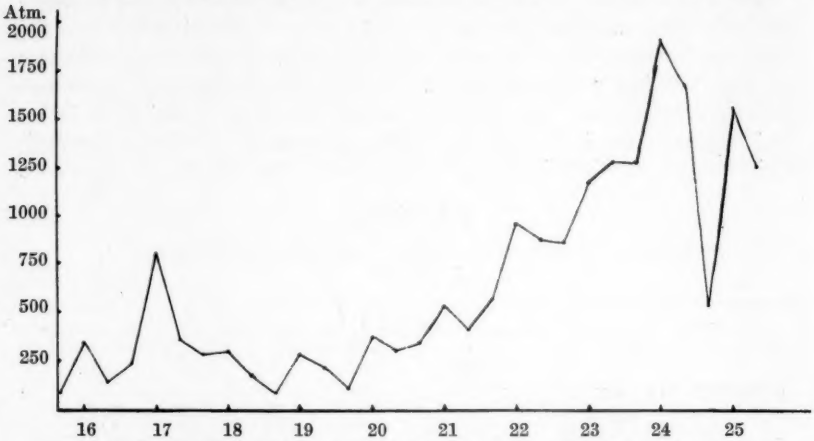

FIG. 3. Diurnal fluctuations of the equilibrium forces with diurnal changes in temperature and atmospheric saturation deficit. Data for Washington, D. C., June, 1932.

much higher in the middle of the day than in the morning or evening. This fact is generally recognized, but no precise quantitative expression of the fact has ever been given.

It is a well known fact, also, that laboratory air is drier than the natural atmosphere, but aside from humidograph or hygrometer readings, the differences between the equilibrium conditions of inside and outside air have never been given quantitative expression. A number of readings were made with a sling psychrometer, first in the laboratory, then in the natural atmosphere. Using MARVIN'S psychrometric tables (**11**) the relative humidity was obtained in each locality. From the humidity readings and the dry bulb temperatures, calculations of the water equilibrium forces were made. Three laboratory readings were 1844, 1532, 1904 atm., respectively, while the corresponding readings outside were 484, 362 and 488 atm. The atmosphere in an oven room was yet much drier than the ordinary laboratory air, a single reading showing an equilibrium tension of 2271 atm. Measured in terms of the equilibrium forces in atmospheres, the laboratory air is about four times, and the oven room air almost five times, as severe as the outside atmosphere.

Organic and inorganic colloidal materials undergo fluctuations in mois-

ture content as the atmospheric conditions change, always toward the establishment of an equilibrium of forces. Seeds which are already in dynamic water equilibrium with the aqueous vapor phase of their environment will begin to increase in weight as soon as the atmosphere increases in relative water content or decreases in temperature; or they will lose weight if the

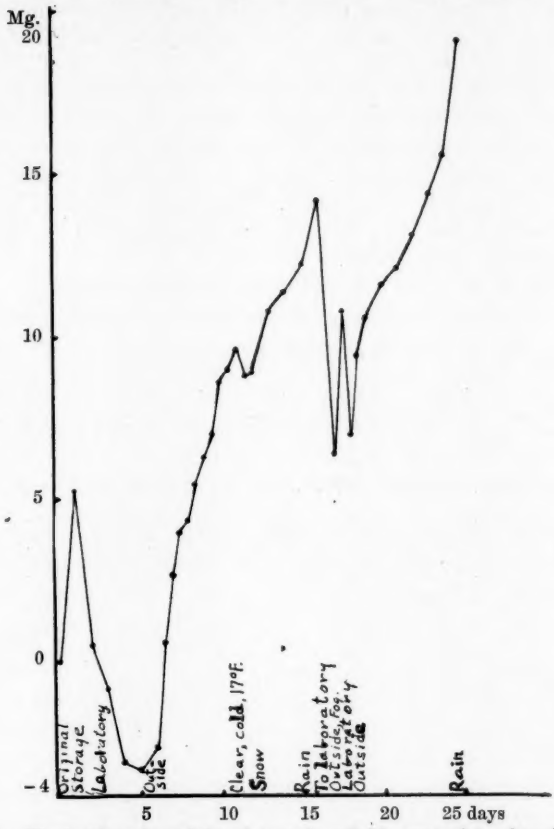

FIG. 4. Fluctuations in weight of organic colloids exposed to changing atmospheric conditions.

air becomes drier or warmer. Figure 4 shows some of the changes in weight of seeds of the Chisholm variety of field corn while subjected to changed environmental conditions. These seeds had been weighed individually some months before they were used in these experiments. At the beginning of the tests, the seeds were about 5 mg. per seed heavier than when first weighed. The preceding weather had been cloudy and rainy, but the seeds were stored

in a laboratory locker. Exposed in the laboratory for a few days, they lost weight. They were then placed out of doors in containers protected from precipitation. The plotted curve indicates the increasing weight as the seeds adjusted themselves to the outside conditions. After some days they were brought into the laboratory for 24 hours, then placed outside for 12 hours, again inside for 12 hours, then outside until they began to fluctuate with the natural environmental changes. It required considerable time for this adjustment, and only the earlier readings are shown in figure 4. It is obvious from this experience that the time lag is important in such work. The rate of diffusional flow of moisture into or out of the seeds must be somewhat proportional to the difference between the vapor pressure of the seed colloids and that of the atmosphere. All kinds of colloidal materials, including soil colloids, behave in this manner. These facts have long been recognized, but it has now become possible to measure and state the equilibrium effects with satisfactory precision.

To demonstrate the practical usefulness of the method, some seeds and some soils were confined in desiccators maintained at a controlled temperature of 20° C., over 4 different concentrations of sulphuric acid, the vapor pressures of which were known. Only an example or two are presented, as more work must be done, especially with the soils, before satisfactory conclusions may be drawn. The concentrations of sulphuric acid solutions used were as follows:

36.5 per cent. sulphuric acid, r.h. 64 per cent., at 20° C. = 595 atm.
50.0 per cent. acid, r.h. 37 per cent., at 20° C. = 1326 atm.
61.75 per cent. acid, r.h. 16 per cent., at 20° C. = 2444 atm.
76.3 per cent. acid, r.h. 3.8 per cent., at 20° C. = 4360 atm.

Marquis spring wheat exposed near the surface of the sulphuric acid for 28 days was found to contain the following percentages of moisture:

Over 36.5 per cent. sulphuric acid, 13.7 per cent. moisture.
Over 50.0 per cent. acid, 9.69 per cent. moisture.
Over 61.75 per cent. acid, 5.89 per cent. moisture.
Over 76.3 per cent. acid, 3.93 per cent. moisture.

Granted that the seeds had attained moisture equilibrium with the acid, one could then say that the force with which the 13.7 per cent. of water is held by the seeds is 595 atmospheres; with further drying, the force increases, and at a water content of 9.69 per cent., the remaining moisture is held with a force of 1326 atmospheres. The grains which have been dried down to a water content of 5.89 per cent., hold their moisture with a force of 2444 atmospheres; and those which had been reduced to 3.93 per cent. water content were holding this small quantity with a force only a little short of 4400 atmospheres.

The results with the soils were not very satisfactory, presumably because

the sassafras series of soils did not vary sufficiently in texture and composition to permit accurate readings in this preliminary attempt. Best results would surely be attainable with soils of higher colloidal content, with greater hygroscopic capacity. At equilibrium over 36.5 per cent. sulphuric acid a sample of sassafras loam contained only 0.76 per cent. of hygroscopic water, which was held by a force of 595 atmospheres; at equilibrium with 50 per cent. acid, the water content of this loam was reduced to 0.47 per cent., which was held by a force of 1326 atm.; at equilibrium with 76.3 per cent. acid only 0.26 per cent. of hygroscopic moisture was left, but it was held with a force of almost 4400 atmospheres. Other tests are planned with heavier soils from the black belt of Alabama. The most difficult problem in connection with such work is to make certain that equilibrium has been actually attained. In this work readings were taken at the end of a 28-day period, chosen arbitrarily, just as in earlier work on soil moisture (**19**) a 15-day period was chosen as appropriate for the attainment of equilibrium relations between soils of different moisture contents and dry seeds which were used for measurement of the resident surface forces of the soils.

Soil and plant responses to the aerial environment

By virtue of the relative humidity and temperature relations which have just been outlined, the atmosphere exerts a profound influence upon the water systems of plants and the soils in which they are rooted. Time will permit but brief reference to some of the more important considerations. In regions where extreme drought prevails during long periods, we see the deserts with their peculiarly adapted vegetation; and any region which suffers prolonged rainless seasons moves rapidly toward desert conditions, a fact painfully illustrated over large areas of the great plains region in recent years.

Soils lose their water by gravitational drainage of free water, by direct evaporation from the soil surface, and by indirect evaporation through the exposed portions of the ground cover vegetation. As long as the soil is well supplied with freely movable capillary water, severe transpiration may take place locally and temporarily without doing much harm to the vegetation, and without producing drought conditions. No land area is ever very far away from drought conditions if the atmosphere is dry and warm for prolonged periods. It was observed in Kansas (**20**) some years ago that when the atmosphere becomes so arid that the evaporation consistently exceeds 0.35 in. per day from a standard evaporation pan, it requires only a few days to develop severe drought conditions for all kinds of plants. As long as the soil is well supplied with moisture, the air itself is kept too humid by evaporation and transpiration to produce that amount of evaporation from the open pan. It is only after the soil has become dry at the surface that the atmosphere can attain conditions causing 0.35 in. of evaporation per day.

By the time this condition is reached, plants are practically limited to the water supply found in the soil mass actually occupied by their root systems. The plants and soils dry down together, but always with the greatest forces for water movement developed in connection with the leaves. In other words, the saturation deficit of the atmosphere is first transferred to the leaf, and is then propagated back through the plant body to the root system, and through the root system into the soil itself. As the soil becomes more and more depleted of water through root absorption and evaporation, the capillary adjustment on the surfaces of the mineral particles and soil colloids becomes slower and slower, until it is finally completely ineffective in the delivery of water to the plant. This point defines the wilting coefficient of the soil, as was pointed out many years ago (**19**). The plant, threatened by relentless evaporation and unyielding soil, does what it can to adjust itself to its diminishing water content. Lower leaves begin to die; or, as in the case of the common poplar, the leaves may be abscissed in great numbers, even in early summer. In cases where the drought is not so severe as to cause death or leaf fall, growth is slowed down, the new leaves are not as well expanded, and because of this fact show a larger number of stomata per unit area. MAXIMOV (**12**) has clearly outlined the stomatal responses of plants to atmospheric conditions. In a brief paper YOCUM (**30**) has recently described the situation with reference to oak trees. Height of insertion of leaves upon the tree does not affect stomatal numbers in the case of mature trees which produce their entire crop of leaves at one time in early spring. Branches that continue to grow and leaf throughout the season, however, bear smaller upper leaves, with increased numbers of stomata per unit area because of the lesser expansion of the new leaves formed during the drier summer season.

In a former paper (**23**) we have discussed the development of colloidal imbibitional forces in the cell walls bordering the intercellular spaces of the leaf, and the conversion of imbibitional forces into osmotic action in the mesophyll cells of the leaf. The development of such forces through saturation deficits is a necessary prerequisite for the ascent of sap. The columns of water will not move upward until the forces are sufficient to lift them and overcome all frictional resistance to movement.

From our studies of eosin absorption by submerged shoots and twigs (**23**) in which the saturation deficit had been largely relieved by provision of a free water supply following submergence, we have concluded that sap ascent is caused by the physical and chemical effects of evaporation on the cell colloids and the forces generated thereby, and is not associated with an active cellular excretion of water at the expense of respiratory energy. Both DIXON (**3**) and BOSE (**1**) thought that there was evidence of such secretion, but we were unable to observe it in saturated tissues. It is believed that DIXON's results rested upon the use of unsaturated tissues, as his results

were readily confirmed by experiments conducted according to DIXON's description of his work. In a recent paper VAN DER PAAUW (13) has criticized our method, and claims that by the use of very sensitive potometers he has demonstrated a long continued intake of water by submerged cut shoots. He thinks eosin may be adsorbed at the lower end of the tracheae as fast as it enters when entry is slow, and that the method thus may fail to record sap rise even when it occurs. This criticism by VAN DER PAAUW is not taken very seriously, because our results contrast rather sharply with the eosin experiments of DIXON which would naturally be subject to the same criticism. Moreover, we found long continued intake in leafless submerged twigs with the eosin method even when entry was very slow. This slow entry of water over long periods was attributed in part to changes in gas volume in the tracheae, possibly slow solution of the gases. I am inclined to think that VAN DER PAAUW's results, which involve only small volumes of water, are also related to changes in gas volumes in the tracheae. VAN DER PAAUW does not find anything in his results to support the secretion hypothesis. He merely objects to our conclusion that water does not enter the shoots after they have been saturated. The decision in this case might hinge upon how one defines saturation. At any rate the facts as far as we know them favor the belief that sap ascent is caused by physical and chemical forces which are generated by evaporation of water from imbibed colloidal structures.

From the data presented in table I it can be seen that it does not require a large saturation deficit to accomplish the work of sap ascent. A leaf-colloid vapor pressure deficit of not over one per cent. will provide a stromogenic tension (7) sufficient to supply water to a height of 75 ft. This is estimated on a basis which allows as much force to overcome friction as to lift the columns of water. It also allows a liberal deduction for the net osmotic pressure of the root cells which must be overbalanced by the net osmotic pressure of the leaf cells. A 2 per cent. vapor pressure deficit would provide a tensional pull sufficient for all vegetation except the tallest trees; and a 3 per cent. deficit would provide stromogenic tension greater than that required for the tallest redwoods and eucalypts.

The saturation deficit of the leaf is not constant, but fluctuates rhythmically. It is low in the early morning as a rule, rises during the middle of the day, and falls again at night. This fluctuation of the imbibitional and osmotic values of leaf cells has been observed by many workers. It is caused by several factors working together. In the first place, the increasing transpiration decreases the volume of the cell, and, by concentrating the solutes of the cell sap, decreases the free water of the cell. At the same time, there is increased sugar manufacture in the mesophyll cells, which tends also to decrease the free water. In addition, JIMBO (8) claims that there is some conversion of starch or other condensation products to simpler soluble materials which helps to emphasize the saturation deficit during the middle of the day.

The movement of water from cell to cell in the leaf is controlled by differences in the net osmotic pressure. This term is used to designate the excess of osmotic pressure over wall pressure. It corresponds to URSPRUNG'S (**27**) suction force. For instance, if the osmotic pressure within the cell is 6 atmospheres, and the wall, through being stretched, is pressing upon the protoplasm with a force of 2 atmospheres, the net pressure of the cell is 4 atmospheres. If an adjoining cell happens to have a lower net pressure, water will move by osmosis (diffusion) from the cell of lower net pressure to the cell of higher net pressure. A gradient of net osmotic pressure extends across the mesophyll cells which lie between the intercellular spaces, where evaporation occurs, and the tracheal conducting system. As cells approach incipient plasmolysis, wall pressure becomes smaller and smaller, until the entire osmotic concentration of the cell expresses itself in the diffusional osmotic movement of water into the cell. The osmotic force of a leaf just at the point of incipient plasmolysis may in extreme cases even in normal life (**6**) reach values as high as 200 atmospheres. It is, however, usually much lower than this. In his studies of prairie plants STODDART (**24**) reports concentrations ranging from 20 to 60 atmospheres at the time the plants were dying from drought.

As water leaves the tracheae in response to these imbibitional and osmotic conditions of the leaf, a stromogenic tension is created in the water columns of the tracheal system. The cohesion of water is very great, and the evidence in favor of the cohesion theory of DIXON is fairly convincing. There are many details, however, that need clarification. The best interests of science will be served if, as has been suggested, it is used as a working hypothesis to be tested in every possible manner. Certainly this theory is better grounded than any of the substitutes that have been offered under the name of sap hydraulics. The inclusion of sorption of water on the tracheal walls as a part of the theory of sap rise does not aid us in the theoretical consideration of this problem, because the same force that sustains the columns or films of water so that they do not run out of the tracheae when the stems are cut, will also oppose the upward movement of the columns with exactly the same force. Moreover, the old tensile film theory of QUINCKE, which was discarded many years ago by DIXON as inadequate, but which has recently been revived by PEIRCE (**14, 29**) and his students, cannot be accepted until it is shown that the reasoning on which it was originally discarded is not sound. That has not been done. Finally, it is difficult to see how a central core of vapor in the tracheae could be of any possible advantage in sap ascent, because there seems to be no way in which traction can be obtained in such a column. While evaporation might occur at one end of the system and condensation at the other, the temperature differences favor evaporation above, and condensation at the lower ends of the tracheae, the reverse of what is demanded. As yet we have no real evidence of such vapor

transfer in sap ascent. Such proposals leave one feeling that they are only expressions of opinion, rather than theories dependent for their validity upon an array of supporting evidence. It seems clear, therefore, that we do not yet have any good substitute for the cohesion theory, nor any sound reasons for discarding it at the present time. It is true that we do not understand just what may be the importance of the pneumatic portions of the tracheal system. The reduced air pressures developed within the pneumatic tracts may contribute in some manner to the ascent of sap. Certainly the sudden chilling of the pneumatic system while it is under severe tension, causes large increases in the rate of water intake by cut branches as measured by potometer readings.

The water deficit which is first established in the leaves of the plant is transmitted to the root system through the action of the stromogenic tension in the cohesive water system of the tracheae. Under the stresses developed, water may simply pass from the living cells of the root into the tracheae because the stromogenic tension exceeds the net osmotic pressure of these cells. Such movement of water would establish a saturation deficit in the interior of the root, which would be extended toward the periphery of the root by a combination of osmotic and imbibitional changes. Just as, in the leaf, gradients exist between tracheae and evaporating cells, so gradients of net osmotic pressure are set up in the roots which lead to the movement of free water from the peripheral cells inward toward the tracheal vessels. As soon as the saturation deficit extends to the epidermal cells of the root, the colloidal walls of these cells begin to absorb water from the surfaces of the soil particles which are in contact with the root absorbing cells. The forces involved in root absorption have been considered in a previous paper (**22**).

There has been some disagreement as to the most active regions of root absorption. Some physiologists have considered the root hairs as the main absorbing organs, others have championed the idea that the young portions of the roots where elongation is most rapid and where hairs have not yet been developed are the most active regions of absorption. Root hairs are then considered to have merely, or at least mainly, an anchorage function. The classical view has been that the root hairs are active absorbers, and specifically adapted by form and chemical constitution for their function. There is hope that the truth may soon be established with reference to this difference of opinion, for ROSENE and LUND (**16**) have described a method by which the absorptive activity of any given region of the root may be measured. The results obtained with onion roots indicate that the most active regions of absorption are not just back of the tip, and not in the most rapidly elongating region, but in a region 40 to 50 mm. back of the apex of the root. They do not say whether these onion roots possessed root hairs. But if roots generally prove to be most active 4 to 5 cm. from the tip, the main absorption is in the region where hairs are well developed, and not yet old.

As long as the soil is well provided with water, the root system maintains a rate of water intake usually sufficient to prevent any serious consequences from transpirational water loss. But if the soil as well as the atmosphere becomes arid, the root system can absorb only the quantity of water that is delivered to its surface by capillary movement of water. When the soil particle surface films reach a certain degree of thinness, capillarity is no longer able to deliver a sufficient amount of water to the roots. Those soil particles which are in actual contact with the root hairs become much drier than other soil particles just beyond the limits of the root hairs. Careful observation shows that there is a sharp line of demarcation between the drier and moister particles, and that capillarity has ceased to function. At this point the plant must wilt, no matter how much change occurs in the imbibition pressures of cell walls and protoplasm, no matter how high osmotic values are produced in the vacuoles of the leaf cells, no matter how great stromogenic tension is developed in the tracheae; water cannot be obtained when it is not available, and permanent wilting and death soon follow. Good xerophytes may live for a long time without much water intake, but they do not grow. It is this ability to withstand severe water deficits without actually being killed that distinguishes the true xerophyte.

In one of my early papers (**19**) is was stated that at the wilting coefficient, the soil is withholding water from the plant by only about four atmospheres, and that the root cells have an osmotic pressure of about eight atmospheres if HANNIG'S (**5**) values are accepted as average. In other words there is a good gradient for water movement into the plant, but no significant intake at the wilting point. In this connection I called attention to work by ROBERTS (**15**) and TRUE (**25**) in which it had been found that plant cells adjust their internal concentrations so as to maintain a few atmospheres of diffusional gradient for water intake. MAXIMOV (**12**) has criticized my interpretation because of the fact that I made no distinction between osmotic concentration and the net pressure. So far as my own work is concerned this criticism is unjustified, however, for my measurements concern plants at the time of permanent wilting, at which time there could not possibly be any wall pressure to deduct from the osmotic pressure. At incipient plasmolysis, when wilting first occurs, the total osmotic pressure, equivalent to the osmotic concentration, is available for work. The criticism may apply, however, to the conclusions concerning the results of ROBERTS and of TRUE in which wall pressures were probably not taken into consideration.

There has been a tendency in recent years to minimize the importance of soil capillarity with reference to water movement in response to root absorption. One difficulty is that the studies of water movement are usually made on disturbed soils. By suitable tunnelling of soils it should be possible to study capillary movements in undisturbed soil masses. The physical structure of disturbed soils is always different from that of natural soils and

could hardly be expected to show normal behavior. This may account for some of the low measurements of capillary water movement obtained by soil scientists. As long as the soil contains moisture above the wilting coefficient one would expect capillary action, more rapid when near the field capacity, and less and less rapid as the soil dries toward the wilting coefficient. In the range of soil moisture between the wilting coefficient and air dry condition, capillarity disappears as a factor. Water movement would then be confined to diffusion of water vapor through the soil from moister to dryer regions, or from warmer to colder regions.

In the moisture range just slightly above and below the wilting coefficient, root extension and development of new root hairs may be important in tapping new water supplies in the soil. In a sense the plant may go after water when water ceases to move adequately toward the plant; but certainly growth of the roots could not continue very actively with the medium of growth close to the wilting coefficient and with the top of the plant suffering large saturation deficit.

Summary

In conclusion one may summarize the relations as follows: the plant body is a mass of organic colloidal materials organized for the processes of living. These colloidal materials cannot function in life unless they are well supplied with water. In addition to colloids and water, there are inorganic ions and molecules, and organic molecules, in solution. The water in the plant is part of the general water system of nature which has a vapor phase in the atmosphere, and both liquid and vapor phases in plants and soils.

The plant body is, under normal conditions of water supply, in a much swollen condition. The colloidal materials are swollen by imbibition, and the organized cells are swollen by osmotic pressure. This swollen condition is subject to the balance between water absorption through one permeable region of the plant (the root), and water loss through another permeable region, the shoot. If loss is greater than gain, swelling decreases because of saturation deficit. If gain is greater than loss at any time during such a period of deficit, the swelling again increases. During the autokinetic phase of plant growth, absorption is on the average more rapid than water loss, because the increasing size of the plant body requires, in addition to the replacement of transpirational loss, more water to satisfy the needs of its newly created protoplasm, cell walls, and vacuoles. This increasing mass of water represents the retentive capacity of the materials for water. During the autostatic phases of the life cycle, water loss exceeds gain as the plant body ripens. This is particularly true of annual plants.

The shrinking of the plant body under evaporational water loss generates colloidal surface forces which are propagated through the plant body, from leaf to stem, from stem to root, from root to soil. The generation and propa-

gation of these forces tend to hasten water absorption as long as the soil possesses enough free water to permit the speeding up of absorption. If the soil too is becoming dry, this same generation of forces in the leaf continues, with more and more shrinkage of colloids and cell vacuoles, and generation of greater and greater forces. The soil keeps on supplying water at rates set by its physical and chemical properties, and by the forces generated on the surfaces of the soil particles themselves. If the process goes far enough, the plant first becomes less active, then ceases to grow, after which it continues to dry out until it and the soil have reached the stage represented by the permanent wilting of the plant and the wilting coefficient stage of soil moisture. Adequate precipitation at any earlier stage than permanent wilting would reverse these processes. The body would begin to swell, the wilted leaves to revive, the meristems to renew active growth, and the plant as a whole to resume normal living. Permanent wilting, if prolonged, usually means death.

The forces of sap ascent reside first of all in the water molecules themselves, which by evaporating from colloidal materials generate the forces for sap ascent. These forces are transformed from imbibitional to osmotic action in the leaves, and express themselves as stromogenic tensions in the hydrostatic and pneumatic vascular tracts of the plant. If not all of the details of sap ascent are clear, at least the main outlines of the cohesion theory seem to be well grounded.

These water relations are important because maximum plant production depends upon the plant's ability, up to the limit of its inherent or hereditary capacity, to gather in materials and create plant substances from them. A thorough understanding of all of the water relations is fundamental to an intelligent management of the plant's environment. The function of this paper is to clarify particularly the relation of the water vapor phase of the atmosphere to the water economy of plants in general.

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STUDIES ON ANTAGONISTIC PHENOMENA AND CATION ABSORPTION IN TOBACCO IN THE PRESENCE AND ABSENCE OF MANGANESE AND BORON

T. R. SWANBACK

(WITH TEN FIGURES)

Introduction

Tobacco is very sensitive to slight variations in cultural or nutritional conditions, and is thus particularly suitable for physiological studies. Most tobacco is ultimately to be burned (smoked), and as a result great interest centers in the burning qualities of the leaf and the relation of these to the ash resulting and to factors governing ash composition. All of the above items in turn are reflected in the growth of the plant. The object of the present investigation was to study, by means of water cultures, the absorption of mineral nutrients. The bases, and factors governing their translocation within the plant, were studied specifically.

Methods

In beginning experiments, a cultural method described by LUNDEGÅRDH (**6**) was employed. Tobacco plants of Havana seed type about four weeks old, were grown in sterile quartz sand with added weak nutrient solution. These were transferred to glass tubes about 45 mm. in diameter, of 200 to 250 ml. capacity, and filled with a measured quantity of a nutrient solution. The plants, one for each vessel, were fastened in a cork stopper 40 mm. in diameter,

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS IN THE POTASSIUM SERIES. CONCENTRATIONS GIVEN IN GRAMS PER LITER

SALTS	CONCENTRATION	A (LOW K CONTENT)						
		CONCENTRATION						
		N	P	K	Ca	Mg	Mn	B
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
$Ca(NO_3)_2 \cdot 4H_2O$	0.847	0.100			0.143			
$NaH_2PO_4 \cdot H_2O$	0.156		0.017					
Variable A B C								
K_2SO_4 .06; .185; .6				0.026				
$MgSO_4 \cdot 7H_2O$	0.308					0.030		
$MnCl_2 \cdot 4H_2O$	0.001						0.0003	
H_3BO_3	0.005							0.0008
Fe-citrate	0.01							
Total		0.100	0.017	0.026	0.143	0.030	0.0003	0.0008

TABLE I—(*Continued*)

COMPOSITION OF NUTRIENT SOLUTIONS IN THE POTASSIUM SERIES. CONCENTRATIONS GIVEN IN GRAMS PER LITER

SALTS	CONCENTRATION	B (MEDIUM K CONTENT)						
		CONCENTRATION						
		N	P	K	Ca	Mg	Mn	B
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
$Ca(NO_3)_2 \cdot 4H_2O$	0.847	0.100			0.143			
$NaH_2PO_4 \cdot H_2O$	0.156		0.017					
Variable A B C								
K_2SO_4 .06; .185; .6				0.082				
$MgSO_4 \cdot 7H_2O$	0.308					0.030		
$MnCl_2 \cdot 4H_2O$	0.001						0.0003	
H_3BO_3	0.005							0.0008
Fe-citrate	0.01							
Total		0.100	0.017	0.082	0.143	0.030	0.0003	0.0008

TABLE I—(*Concluded*)

COMPOSITION OF NUTRIENT SOLUTIONS IN THE POTASSIUM SERIES. CONCENTRATIONS GIVEN IN GRAMS PER LITER

SALTS	CONCENTRATION	C (HIGH K CONTENT)						
		CONCENTRATION						
		N	P	K	Ca	Mg	Mn	B
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
$Ca(NO_3)_2 \cdot 4H_2O$	0.847	0.100			0.143			
$NaH_2PO_4 \cdot H_2O$	0.156		0.017					
Variable A B C								
K_2SO_4 .06; .185; .6				0.26				
$MgSO_4 \cdot 7H_2O$	0.308					0.030		
$MnCl_2 \cdot 4H_2O$	0.001						0.0003	
H_3BO_3	0.005							0.0008
Fe-citrate	0.01							
Total		0.100	0.017	0.26	0.143	0.030	0.0003	0.0008

which was supported by a wire folded over the edge of the tube. Blackened cardboard folded around the tubes excluded light from the roots. The tubes, in triplicate for each treatment, were placed in a rack and transferred to a photothermostat (**6**). The nutrient solutions are given in tables I and II and the cultural variations in tables III, IV, and VII.

In all of the solutions purest Kahlbaum salts were used. In order to obtain a true, clear solution where it was necessary to employ calcium sulphate, a saturated solution of this salt was used (solubility in water of room temperature about 0.23 per cent.). The solution was analyzed as to Ca content and a quantity was added sufficient to meet the Ca requirements of the different

nutrient solutions. The reaction of solutions was equalized as nearly as possible at the beginning of the experiment. It varied slightly from a pH of 5.2 to 5.4. The solutions were changed weekly and plants were harvested after 45 days.

In later experiments, seedlings were grown in a soil flat. After six weeks they were transferred to one-liter Jena glass beakers each containing a measured quantity of nutrient solution. The beakers had been treated for about a month with a weak HCl solution. They were then thoroughly rinsed

TABLE II

COMPOSITION OF NUTRIENT SOLUTIONS IN THE CALCIUM SERIES. CONCENTRATIONS IN GRAMS PER LITER

SALTS	CONCENTRATION	A (LOW Ca CONTENT)						
		N	P	K	Ca	Mg	Mn	B
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
$Ca(NO_3)_2 \cdot 4H_2O$	0.25	0.03			0.042			
$NaH_2PO_4 \cdot H_2O$	0.156		0.017					
KNO_3	0.214	0.03		0.082				
$NaNO_3$	0.037	0.006						
$Mg(NO_3)_2 \cdot 6H_2O$	0.32	0.034				0.03		
$MgSO_4 \cdot 7H_2O$								
$MnCl_2 \cdot 4H_2O$	0.001						0.0003	
H_3BO_3	0.005							0.0008
K_2SO_4								
Fe-citrate	0.01							
$Na_2SO_4 \cdot 10H_2O$	0.32							
$CaSO_4 \cdot 2H_2O$								
Total		0.100	0.017	0.082	0.042	0.03	0.0003	0.0008

TABLE II (*Continued*)

COMPOSITION OF NUTRIENT SOLUTIONS IN THE CALCIUM SERIES. CONCENTRATIONS IN GRAMS PER LITER

SALTS	CONCENTRATION	B (MEDIUM Ca CONTENT)						
		N	P	K	Ca	Mg	Mn	B
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
$Ca(NO_3)_2 \cdot 4H_2O$	0.847	0.100			0.143			
$NaH_2PO_4 \cdot H_2O$	0.156		0.017					
KNO_3								
$NaNO_3$								
$Mg(NO_3)_2 \cdot 6H_2O$								
$MgSO_4 \cdot 7H_2O$	0.308					0.03		
$MnCl_2 \cdot 4H_2O$	0.001						0.0003	
H_3BO_3	0.005							0.0008
K_2SO_4	0.185			0.082				
Fe-citrate	0.01							
$Na_2SO_4 \cdot 10H_2O$								
$CaSO_4 \cdot 2H_2O$								
Total		0.100	0.017	0.082	0.143	0.03	0.0003	0.0008

TABLE II (*Concluded*)

COMPOSITION OF NUTRIENT SOLUTIONS IN THE CALCIUM SERIES. CONCENTRATIONS IN GRAMS PER LITER

SALTS	CONCENTRATION	C (HIGH CA CONTENT)						
		N	P	K	Ca	Mg	Mn	B
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
$Ca(NO_3)_2 \cdot 4H_2O$	0.847	0.100			0.143			
$NaH_2PO_4 \cdot H_2O$	0.156		0.017					
KNO_3								
$NaNO_3$								
$Mg(NO_3)_2 \cdot 6H_2O$								
$MgSO_4 \cdot 7H_2O$	0.308					0.03		
$MnCl_2 \cdot 4H_2O$	0.001						0.0003	
H_3BO_3	0.005							0.0008
K_2SO_4	0.185			0.082				
Fe-citrate	0.01							
$Na_2SO_4 \cdot 10H_2O$								
$CaSO_4 \cdot 2H_2O$	1.13				0.26			
Total		0.100	0.017	0.082	0.403	0.03	0.0003	0.0008

with distilled water to eliminate, as far as possible, contamination from the glass. The beakers were covered with blackened cardboard and on top of each was placed a sheet of black-varnished galvanized iron, in the center of which was a hole fitted with a 5-cm. cork stopper for holding the plant. Before transfer from the soil flat, each plant was thoroughly rinsed in distilled water and placed in a beaker. Non-absorptive cotton was packed around the stem of each plant. Each nutrient variant was set up in quadruplicate. The beakers were placed in artificially illuminated photothermostatically controlled chambers, described by LUNDEGÅRDH (**6**). The plants were allowed to grow for 60 days. Nutrient solutions were changed in the middle of the growth period. At this time a slight precipitation was evident in high-calcium solutions.

Nitrate nitrogen was determined in the solutions by the disulphonic acid method, and phosphorus by the phosphoceruleomolybdic acid method. Solutions, salts, and plant materials were analyzed for cations by the quantitative spectrographical methods described by LUNDEGÅRDH (**6**).

Experimental results

THE EFFECT OF VARYING QUANTITIES OF CA ON GROWTH AND ON THE ABSORPTION OF POTASSIUM, CALCIUM, AND MAGNESIUM

Data for plant growth and cation absorption are given in tables III and IV; the first represents results from an experiment set up under the first procedure outlined under "Methods"; the second, under the revised procedure.

The data in table III show a marked response in growth as measured by

TABLE III

DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS WITH VARYING AMOUNTS OF CALCIUM,* EXPERIMENT 1

TREATMENT AND PLANT PARTS	DRY WEIGHT		ASH ANALYSIS					
			K		Ca		Mg	
	PLANT	RELATIVE	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART
	gm.		*m. mol*	*m. mol*	*m. mol*	*m. mol*	*m. mol*	*m. mol*
Low Ca								
Stems, leaves	0.416		1.240	0.516	0.259	0.108	0.330	0.137
Roots	0.110		1.030	0.113	0.052	0.016	0.285	0.031
Total plant	0.526	46		0.629		0.124		0.168
Medium Ca								
Stems, leaves	0.845		0.810	0.684	0.472	0.399	0.135	0.114
Roots	0.300		0.690	0.207	0.085	0.026	0.180	0.054
Total plant	1.145	100		0.891		0.425		0.168
High Ca								
Stems, leaves	1.010		0.760	0.768	0.426	0.430	0.086	0.087
Roots	0.370		0.720	0.266	0.089	0.033	0.202	0.075
Total plant	1.380	120		1.034		0.463		0.162

* Low Ca = 1.05 millimols per liter.
Medium Ca = 3.50 " " "
High Ca = 10.2 " " "

TABLE IV

DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS WITH VARYING AMOUNTS OF CALCIUM,* EXPERIMENT 1

TREATMENT AND PLANT PARTS	DRY WEIGHT		ASH ANALYSIS					
			K		Ca		Mg	
	PLANT	RELATIVE	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART
	gm.		*m. mol*	*m. mol*	*m. mol*	*m. mol*	*m. mol*	*m. mol*
Low Ca								
Leaves	1.26		1.47	1.85	0.68	0.86	0.55	0.69
Stems	0.31		0.82	0.43	0.12	0.62	0.08	0.04
Roots	0.21							
Total plant	1.87	40		2.28		1.48		0.73
Medium Ca								
Leaves	2.47		1.73	4.27	1.06	2.62	0.27	0.67
Stems	1.35		1.34	1.81	0.12	1.62	0.08	0.11
Roots	0.45		0.68	0.31	0.21	0.10	0.20	0.09
Total plant	4.27	100		6.39		4.34		0.87
High Ca								
Leaves	7.28		1.27	9.25	0.93	6.77	0.20	1.46
Stems	6.39		0.59	3.77	0.14	0.89	0.02	0.13
Roots	1.71		0.38	0.65	0.14	0.24	0.20	0.34
Total plant	15.38	360		13.67		7.90		1.93

* Low Ca = 1.05 millimols per liter.
Medium Ca = 3.50 " " "
High Ca = 10.2 " " "

dry weight with increment of Ca in the nutrient solution. Dry matter production with the medium supply is more than double that of the low supply, although the high supply produced only 20 per cent. more dry matter than the medium. Stems and leaves increased in about the same proportion as the roots. In figure 1 a graphical presentation of the relation between growth, Ca supply, and Ca absorption is given.

Of greater interest, however, is the absorption of certain elements as reflected by the ash analyses. Absorbed Ca increased 45.3 per cent. per unit of dry matter above that for "low Ca" with the addition of about three times as much Ca as in the low solution. Further addition of calcium, however, lowered the Ca content per unit dry matter in the stems and leaves, although the total per plant increased but not in proportion to added Ca.

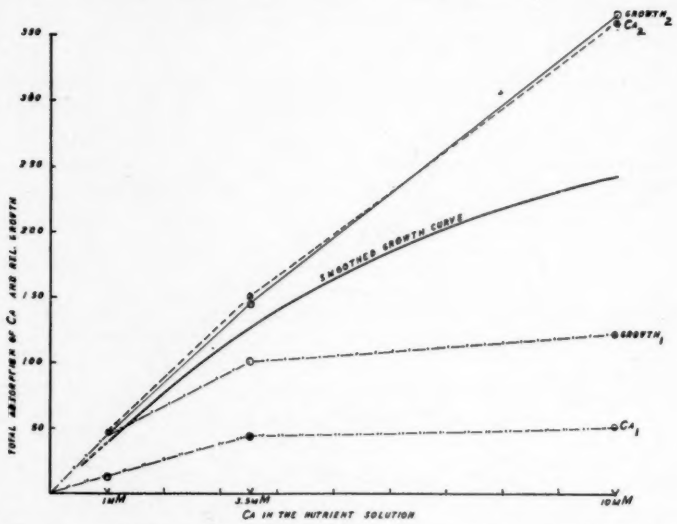


FIG. 1. Graphs showing the relation of growth to calcium supplied in the nutrient solution. The smoothed growth curve shows the general trend of experiments 1 and 2.

Considering the percentage content of Ca in the stems and leaves only, with "medium" as 100, the relationship of the low, medium, and high cultures was about 55, 100, and 90 respectively. The corresponding figures for K were about 153, 100, and 94 respectively, and for Mg, 244, 100, and 64. A low Ca supply in the nutrient medium causes a variation in the absorption of the three bases which occur in the plant in the order Mg > K > Ca, while at a high Ca supply the variation is slight and the order of occurrence is K > Ca > Mg. Since the order of Mg is directly reversed and the only

variable to account for this change is Ca, the antagonism Ca : Mg is plainly established.

A slight K : Ca antagonism is suggested which is emphasized, however, in following experiments where K is the variable. No Ca : K antagonism is indicated in figure 2 where the K content shows a considerable drop at medium Ca supply. With low Ca supply (1 millimol), and with K at 2.1 millimols, any antagonism that Ca may have possessed for K was entirely obscured by the differences in concentration of the two elements.

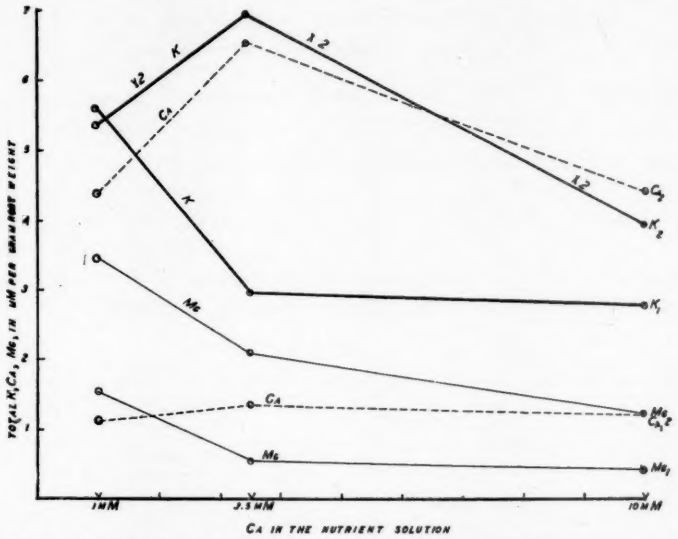


FIG. 2. Graphs showing the relation of total potassium, calcium, and magnesium absorbed per gram of root weight to varying supply of calcium in the nutrient solutions (experiments 1 and 2).

In the second experiment, table IV, with a growing period of 60 instead of 45 days, the high Ca supply produced a considerably higher yield of dry matter than shown in table III. The resulting growth was approximately proportional to Ca supplied in the nutrient solution as evident from relative weights and from figure 1. The data show that stem production was most influenced by Ca, followed by roots, with leaves least influenced by this element. These data also show that Ca noticeably affected the absorption of Mg, with no antagonistic effect on K. A slight pseudo-antagonism Ca : K is suggested at the highest Ca level, more plainly shown in figure 2.

The interrelationship of the three bases, K, Ca, and Mg, is similar to that in the first experiment; for K the relative figures for a low, medium, and

high Ca supply is 78, 100, and 60; for Ca, 64, 100, and 57; and for Mg, 275, 100, and 44 respectively. The order for the three bases at the low Ca supply is Mg > K > Ca, and at the high Ca supply, K > Ca > Mg, which is identical with the results noted above.

The total absorption of the three bases at a low, medium, and high Ca supply, expressed by ratios with low Ca as 1, was: K, 1, 2.8, and 6.0; Ca, 1, 2.9, and 5.3; and Mg, 1, 1.2, and 2.6. With one exception, the absorption of the three bases progressively increased with increased Ca supply and, incidentally, dry matter production increased. The growth curve shown in figure 1 runs almost parallel to the curve of total Ca absorption. A smoothed growth curve shows the general trend of response to Ca as a growth factor.

The effect of varying quantities of potassium on growth and on absorption of potassium, calcium, and magnesium

In the second series of experiments K was varied and the resulting data are given in tables V and VI. A set of cultures containing no K was also included; these plants lived through the growing period but showed no progress in growth. In a trial without Ca, the plants soon died. In this comparison it is evident that the plants grown without K were able through transfer of K to progressively younger parts to maintain life, while the

TABLE V

Dry weights and ash analyses of tobacco plants grown in nutrient solutions with varying amounts of potassium,* experiment 1

Treatment and plant parts	Dry weight		Ash analysis					
			K		Ca		Mg	
	Plant	Relative	Per gram	Per plant part	Per gram	Per plant part	Per gram	Per plant part
	gm.		*m.mol*	*m.mol*	*m.mol*	*m.mol*	*m.mol*	*m.mol*
No K	0.060	5.2	Trace		0.770		0.390	
Low K								
Stems, leaves	0.720		0.480	0.346	0.644	0.464	0.152	0.109
Roots	0.280		0.460	0.129	0.069	0.193	0.240	0.067
Total plant	1.000	87.3		0.475		0.657		0.176
Medium K								
Stems, leaves	0.845		0.810	0.684	0.472	0.399	0.135	0.114
Roots	0.300		0.690	0.207	0.085	0.026	0.180	0.054
Total plant	1.145	100.0		0.891		0.425		0.168
High K								
Stems, leaves	0.940		1.530	1.438	0.086	0.081	0.088	0.083
Roots	0.250		1.250	0.313	0.090	0.023	0.103	0.025
Total plant	1.190	104.0		1.751		0.104		0.108

* Low K = 0.66 millimols per liter.
Medium K = 2.10 " " "
High K = 6.66 " " "

no-Ca plants succumbed from failure to replenish the Ca. As seen in table V, only a trace of K was found in the plant material when this element was lacking in the solution, while Ca and Mg contents were the highest in this group of cultures.

With varying amounts of K in the nutrient solution (table V and fig. 3) the production of dry matter varies much less than in the case of Ca. From data in table V it is calculated that K increased 40.7 per cent. per unit of dry

TABLE VI

DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS WITH VARYING AMOUNTS OF POTASSIUM,* EXPERIMENT 2

TREATMENT AND PLANT PARTS	DRY WEIGHT		ASH ANALYSIS					
			K		Ca		Mg	
	PLANT	RELATIVE	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART
	gm.		*m.mol*	*m.mol*	*m.mol*	*m.mol*	*m.mol*	*m.mol*
Low K								
Leaves	8.70		0.50	4.35	1.39	12.09	0.39	3.39
Stems	6.65		0.26	1.73	0.24	1.60	0.13	0.86
Roots	1.97		0.13	0.26	0.11	0.22	0.44	0.87
Total plant	17.31	320		6.34		13.91		5.12
Medium K								
Leaves	2.47		1.73	4.27	1.06	2.62	0.27	0.67
Stems	1.35		1.34	1.81	0.12	1.62	0.08	0.11
Roots	0.45		0.68	0.31	0.21	0.10	0.20	0.09
Total plant	4.27	100		6.39		4.34		0.87
High K								
Leaves	4.89		2.10	10.27	0.80	3.91	0.33	1.61
Stems	3.59		1.38	04.95	0.09	0.32	0.05	0.18
Roots	0.93		1.28	01.19	0.17	0.16	0.17	0.16
Total plant	9.41	220		16.41		4.39		1.95

* Low K = 0.66 millimols per liter.
Medium K = 2.10 " " "
High K = 6.66 " " "

matter when about three times as much K was added for "medium K" as for "low K." Although further additions of K increased K per unit of dry weight, this latter did not respond to further additions, notwithstanding excessive absorption of this element. The relative content of the three bases in stems and leaves at a low, medium, and high K supply was for K: 60, 100, and 190; for Ca: 140, 100, and 18; and for Mg: 113, 100, and 65. Thus, at the low K supply the order in which the bases were absorbed was: Ca > Mg > K; and for the high K supply, K > Mg > Ca. Since Mg kept its place in the rank and since K was the only variable, the antagonism K: Ca is suggested.

There is an apparent pseudo-antagonism, Ca: K at the low K supply (0.66

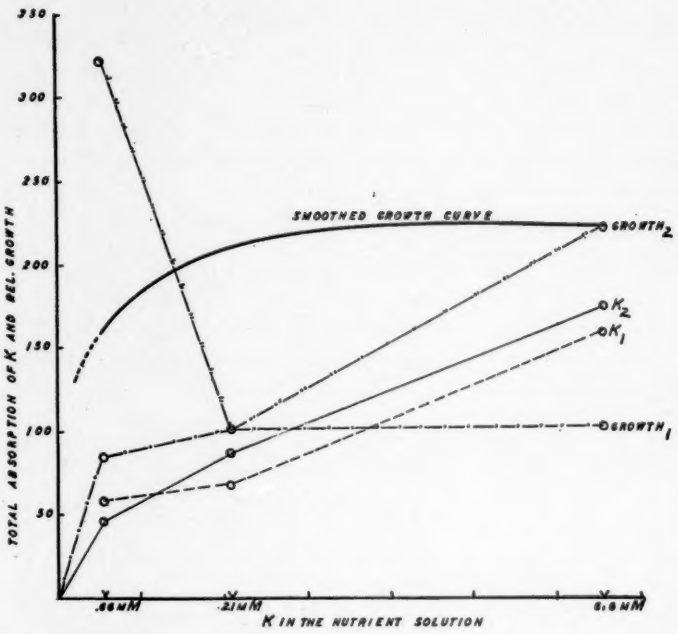


FIG. 3. Graphs showing the relation of total potassium to growth affected by varying the potassium in the nutrient solutions.

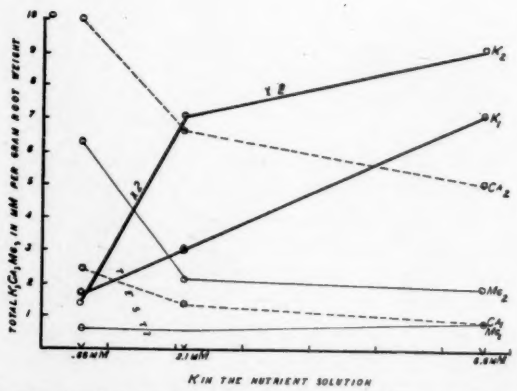


FIG. 4. Graphs showing the relation of total potassium, calcium, and magnesium absorbed per gram of root weight to varying supply of potassium in the nutrient solutions.

millimols per liter), while Ca was present at a concentration of 3.5 (fig. 4). Obviously, a true antagonism did not occur if this instance is compared with the one of medium K supply (2.1 millimols), with Ca constant as above. This K concentration caused a considerable drop in the Ca absorption. Therefore, Ca cannot be considered antagonistic to K although pseudo-antagonism is suggested when the concentration of Ca is vastly higher than that of K.

In the revised experimental procedure, the results of which are given in table VI, K had no direct influence on dry matter production at the concentrations used. In fact, the highest relative weight was produced with the lowest addition of K. It appears that a somewhat lower concentration of K would have been sufficient to produce normal growth.

With the increasing K supply in the nutrient solution, K progressively increased and Ca decreased, while Mg was only slightly affected. The interrelationship of the bases as previously computed is for K: 24, 100, and 109; for Ca: 154, 100, and 69; and for Mg: 130, 100, and 95. At a low K supply the order of base absorption is $Ca > Mg > K$; at a high K supply, $K > Mg > Ca$. This ranking coincides with results of the previous experiment.

Effect of manganese on growth and on the absorption of potassium and calcium

In the absence of Mn in the nutrient solution, deficiency symptoms of this element were pronounced at "high Ca," less at medium, and not noticeable at a "low Ca" supply. The symptoms first occur on the younger leaves (not

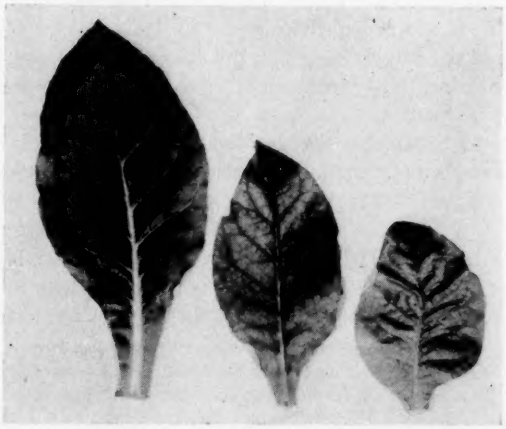


Fig. 5. Leaves from tobacco plants grown (to the left) with manganese and (the two to the right) without manganese.

the youngest) in the form of a chlorosis between the veins and resembles Mn toxicity (6) which, however, appears first at the growing point. The checkered appearance is not as firmly manifested as in the case of the toxicity. In some cases the leaves were crinkled and the size reduced (fig. 5). The roots at "high Ca" without Mn were like those described by McMURTREY (9), *i.e.*, "They did not show many branches but those they had were relatively long." The roots of these cultures were the only ones to reach the bottom of the 1000-ml. containers.

Symptoms of Mn deficiency were very pronounced in "low K" plants, less in "medium," and not noticeable in "high K" plants. The effects with K were thus in reverse order from those with Ca which suggests that Ca is antagonistic to Mn, while K is indifferent.

The data given in table VII show that the presence and absence of Mn (0.0054 millimol per liter) produced vast differences in dry matter in certain instances. With a low Ca supply dry matter in the absence of Mn was six times that obtained where Mn was present. At high Ca supply conditions were reversed so that about 3.5 times as much dry matter was produced in the presence of Mn as in its absence. These results suggest that at a low Ca supply without Mn, there being no antagonism of Mn toward Ca, the Ca could be utilized to the limit. At the high Ca supply the antagonistic action of Mn on Ca was not sufficient to prevent absorption of Ca.

The data of table VII show similar results for "high Ca" and "low K." The latter without Mn resembles "high Ca" with Mn. The presence or absence of Mn at high supply had little influence on growth, suggesting that the antagonism K : Ca obscured the effects of the absence of the essential Mn. The slightly larger weight where Mn was absent might suggest that an antagonism Mn : K was responsible.

The result of these experiments on the absorption of bases in the absence of Mn cannot be considered conclusive since the normal functions of the plant were obviously disturbed. The data of table VIII suggest, however, that Ca is antagonistic to Mn and that Mn is antagonistic to K.

The antagonistic phenomena in relation to Mn are more clearly shown from the standpoint of Mn absorption and translocation by varying the supply of Ca and K in the nutrient solution. Data from such an experiment are given in table VIII. The mobility of Mn, or its relative translocation from the roots to other parts of the plant, may be expressed by a ratio of the Mn content found in stems[1] and leaves and that found in roots. This

[1] No Mn was detected in the stems, when analyzed separately, irrespective of presence of Mn in the nutrient solution. This may be explained on the basis that there would not at any one time be a measurable amount present in the stem where Mn apparently is not stored. It suggests, moreover, that Mn is not a part of compounds formed in the leaf and later translocated into the stems. This is in line with the viewpoint that Mn acts as a catalytic agent.

TABLE VII

DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS WITH VARYING QUANTITIES OF POTASSIUM AND CALCIUM; AND WITH AND WITHOUT MANGANESE*

TREATMENT AND PLANT PARTS	DRY WEIGHT				ASH ANALYSIS					
					CONCENTRATION PER GRAM					
	PLANT		RELATIVE		K		CA		MG	
	MN		MN		MN		MN		MN	
	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT
	gm.	*gm.*			*m.mol*	*m.mol*	*m.mol*	*m.mol*	*m.mol*	*m.mol*
Low Ca										
Lower leaves	1.26	5.55				0.98		0.99		0.65
Top "					1.47	1.19	0.72	0.36	0.55	0.51
Stems	0.31	3.33				1.11		0.06		0.09
Roots	0.21	1.42			0.82	0.49	0.14	0.08	0.08	0.31
Total plant	1.78	10.30	40	240	2.28	10.45	1.48	4.20	0.73	3.96
High Ca										
Lower leaves					1.57	2.31	1.54	1.71	0.19	0.34
Medium "	7.28	2.85			1.10		0.88		0.23	
Top "					1.15	1.54	0.38	0.66	0.18	0.30
Stems	6.39	2.75			0.59	1.95	0.14	0.39	0.02	0.10
Roots	1.71	0.79			0.38	0.43	0.13	0.19	0.20	0.54
Total plant	15.38	6.39	368	150	13.67	11.20	7.90	4.67	1.93	1.59
Low K										
Lower leaves	8.70	3.45			0.37	0.63	1.77	1.87	0.44	0.51
Top "					0.53	1.25	0.41	0.61	0.19	0.41
Stems	6.65	2.04			0.26	0.65	0.24	0.29	0.13	0.21
Roots	1.97	0.57			0.13	0.17	0.11	0.30	0.44	0.72
Total plant	17.31	6.06	405	142	6.34	4.67	13.91	5.05	5.12	2.42
High K										
Lower leaves	4.89	6.52			2.20	2.95	1.22	0.96	0.28	0.19
Medium "					2.25	3.11	0.51	1.52	0.26	0.36
Top "					1.75	2.27	0.28	0.51	0.36	0.23
Stems	3.59	2.45			1.38	2.03	0.09	0.29	0.05	0.10
Roots	0.93	0.72			1.28	1.84	0.17	0.22	0.17	0.18
Total plant	9.41	9.70	220	227	16.41	24.38	4.39	7.43	1.95	2.08

* Mn concentration 0.0054 millimol per liter.

TABLE VIII

MANGANESE CONTENT IN MILLIMOLS PER GRAM DRY WEIGHT OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS WITH VARYING AMOUNTS OF CALCIUM AND POTASSIUM; AND TRANSLOCATION QUOTIENTS FOR MANGANESE

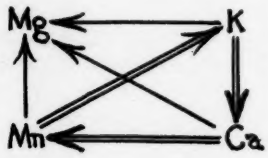
TREATMENT AND PLANT PARTS	GROWTH PERIODS			
	MANGANESE X 0.001 PER GRAM		MANGANESE TRANSLOCATION QUOTIENT	
	45 DAYS	60 DAYS	45 DAYS	60 DAYS
	m.mol	*m.mol*		
Low Ca				
Leaves, stems	0.74	0.75	0.91	1.25
Roots	0.82	0.60		
Medium Ca				
Leaves, stems	0.25	0.36	0.70	0.07
Roots	0.36	5.00		
High Ca				
Leaves, stems	0.16	0.33	0.61	0.06
Roots	0.26	5.60		
Low K				
Leaves, stems	0.22	0.20	0.43	0.10
Roots	0.51	2.10		
Medium K				
Leaves, stems	0.25	0.36	0.70	0.07
Roots	0.36	5.00		
High K				
Leaves, stems	0.30	0.28	0.61	0.09
Roots	0.49	3.20		

ratio is designated the translocation quotient (Tq). Data from translocation quotients in table VIII show that the least resistance to Mn absorption is offered by "low Ca" and the highest by "high Ca." Since "medium Ca" is intermediate a rather definite antagonism Ca : Mn is suggested.

With the low, medium, and high K supply there is no regularity in Mn translocation quotient relations. At the "low K" level, however, the translocation quotient even lower than that at "high Ca" suggests that increased Ca absorption incident to "low K" is responsible for reduced Mn translocation. In this connection it should be recalled that the relative absorption of Ca was greater at "low K" than at "high Ca."

Although no special Mg test similar to those with Ca and K was undertaken, the analytical data in table VI and VII indicate that the absorption of this element is depressed under low Ca and low K supplies in the presence of Mn. It appears, however, that the antagonism Ca : Mn is stronger than that of Ca : Mg. Therefore, it may be concluded that the rank of absorption may be Mn > Mg; K > Ca.

Graphically this may be visualized in the following way:



The arrows point in the direction of the antagonism, the heavy lines suggesting a strong antagonism, the lighter lines a weaker antagonism. Potassium is thus antagonized only by Mn which is in line with the excessive absorption of this latter element, when other elements do not provide a counter antagonism. According to this concept Mg is antagonized by all three of the other elements. Its absorption must be controlled and is possible only because of the counter antagonistic effects of the other elements on one another.

THE EFFECT OF BORON ON GROWTH AND ON THE ABSORPTION OF CALCIUM AND POTASSIUM

In a previous paper (**17**) the writer has shown that boron is essential for normal growth of tobacco plants. In the present work these findings have been confirmed. Deficiency symptoms (arrested top growth, crinkled and brittle leaves) occurred (fig. 6) soon after the experiment was begun

FIG. 6. Boron deficiency ("high Ca").

at low-Ca and -K and high-K supplies. At a high Ca supply the symptoms did not appear until the middle of the growing period. The reason for this may be that minute traces of B (too small to detect by any known analytical method) adhered to the Ca salt used to obtain the high Ca supply. Boron content was apparently sufficient to sustain growth for a certain length of time.

TABLE IX

DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANT GROWN IN NUTRIENT SOLUTIONS WITH VARYING QUANTITIES OF POTASSIUM AND CALCIUM; AND WITH AND WITHOUT BORON

TREATMENT AND PLANT PARTS	DRY WEIGHT				ASH ANALYSIS					
					CONCENTRATION PER GRAM					
	PLANT		RELATIVE		K		Ca		Mg	
	B		B		B		B		B	
	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT
	gm.	*gm.*			*m. mol*	*m. mol*	*m. mol*	*m. mol*	*m. mol*	*m. mol*
Low Ca										
Lower leaves	1.26	0.71			1.47	1.23	0.72	0.68	0.55	0.78
Top "										
Stems	0.31	0.23			0.82	0.50	0.14	0.12	0.08	0.14
Roots	0.21									
Total plant	1.78	0.94	40	20	2.28	0.99	1.48	0.51	0.73	0.58
High Ca										
Lower leaves					1.57		1.54		0.19	
Medium "	7.28	3.31			1.10	1.30	0.88	1.35	0.23	0.17
Top "		1.95			1.15	1.69	0.38	0.91	0.18	0.23
Stems	6.39	1.35			0.59	1.13	0.14	0.52	0.02	0.07
Roots	1.71	0.77			0.38	0.74	0.13	0.29	0.20	0.03
Total plant	15.38	7.38	368	170	13.67	9.70	7.90	7.15	1.93	1.12
Low K										
Lower leaves	8.70	0.84			0.37	1.05	1.77	1.05	0.44	0.47
Top "					0.53		0.41		0.19	
Stems	6.65	0.20			0.26	0.38	0.24	0.42	0.13	0.23
Roots	1.97				0.13		0.11		0.44	
Total plant	17.31	1.04	405	20	6.34	0.96	13.91	0.97	5.12	0.44
High K										
Lower leaves					2.20		1.22		0.28	
Medium "	4.89	1.94			2.25	2.22	0.51	1.49	0.26	0.36
Top "		0.62			1.75	1.77	0.28	0.37	0.36	
Stems	3.59	0.84			1.38	1.77	0.09	0.15	0.05	0.09
Roots	0.93	0.25			1.28	0.53	0.17	0.22	0.17	0.44
Total plant	9.41	3.65	220	80	16.41	6.92	4.39	3.31	1.95	1.11

Data from the boron experiment are found in table IX. In studying the ash analyses it is found that the presence or absence of B in the nutrient solution had a consistent effect on the absorption of the bases; in some instances, the plants without B accumulating more bases per unit of dry weight than those with B supplied. When this occurred it apparently was caused by retarded growth since normal plants were not obtained in the absence of B.

From the difference between the amounts applied and the amounts found at renewal of solutions and at the termination of the experiment, the amount of nitrate nitrogen absorbed by the plants was estimated. The absorption relationship only between treatments that showed great differences are considered. The data are given in table X. Invariably, more nitrates were

TABLE X

NITRATE NITROGEN ABSORBED IN MILLIMOLS PER GRAM DRY MATTER

TREATMENT	NITRATE-NITROGEN
	m. mol.
Low Ca with B	12.8
" Ca without B	25.8
High Ca with B	3.3
" Ca without B	7.6
Low K with B	3.9
" K without B	21.4
High K with B	4.0
" K without B	11.0

absorbed in the absence of B. The smallest difference appears in "high Ca." This is reflected in the small differences in cation absorption of the high-Ca treatments with and without B (table IX).

THE EFFECT OF VARYING QUANTITIES OF POTASSIUM AND CALCIUM ON THE ABSORPTION OF IRON, SODIUM, AND PHOSPHORUS

From the data in table XI it is suggested that the absorption of Fe is retarded by Ca. The approximate "Tq" for Fe is 1.0 at "low Ca," where in the cultures with larger amounts of Ca, the quotient is lowered to 0.20 or less. The accumulation of Fe in the roots in the presence of larger quantities of Ca might be due to a diminution of the pores in the cell walls which Ca is known to produce. It is possible also that insoluble Fe-compounds are formed and, therefore, not translocated away from the roots.

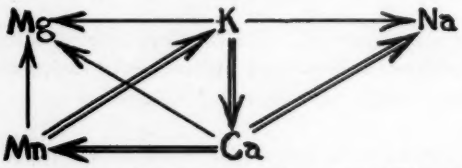
The absorption of Na was retarded more by Ca than by K. The "Tq" of Na would probably decrease rapidly with increasing supply of Ca, and increase with increasing K. It might be gathered that there is a stronger antagonism Ca : Na than K : Na.

TABLE XI

THE CONTENT OF IRON, SODIUM, AND PHOSPHORUS IN MILLIMOLS PER GRAM DRY WEIGHT OF PLANTS GROWN WITH VARYING QUANTITIES OF CALCIUM AND POTASSIUM

TREATMENT	Fe	Tq_{Fe}	Na	Tq_{Na}	P	Tq_P
	m.mol		*m.mol*		*m.mol*	
Low Ca						
Stems, leaves	0.005	1.0	0.29	0.42	0.14	0.30
Roots	0.005		0.69		0.46	
Medium Ca						
Stems, leaves	0.005	0.20	0.05	0.45	0.14	0.87
Roots	0.023		0.11		0.16	
High Ca						
Stems, leaves	0.003	0.15	trace		0.15	1.07
Roots	0.020		0.07		0.14	
Low K						
Stems, leaves	0.005	0.20	0.05	0.30	0.19	0.82
Roots	0.022		0.17		0.23	
Medium K						
Stems, leaves	0.005	0.20	0.05	0.45	0.14	0.87
Roots	0.023		0.11		0.16	
High K						
Stems, leaves	0.005	0.20	0.05	1.00	0.17	1.06
Roots	0.024		0.05		0.16	

Adding Na to the graphic presentation of interrelated antagonisms given previously suggests the following relationship:



It is difficult to explain why the absorption of Na is dominated by Ca and K. It is possible that the greater hydrolization of Na salts plays a part. It is possible also that after Ca and K have entered the stems and leaves they may exercise their antagonistic action toward Na. It is doubtful that insoluble compounds of Na accumulate in the roots.

Phosphorus differs from the other elements discussed in that it is absorbed as an anion. The P content per unit of dry weight varies somewhat, yet its "Tq" appears to be much more significant, varying directly with Ca and to a lesser degree, with K. It appears that Ca facilitates the translocation of P. When K is present in greater abundance, it likewise aids somewhat in this function.

Discussion

In figure 7 are given the smoothed curves of absorption of K, Ca, and Mg

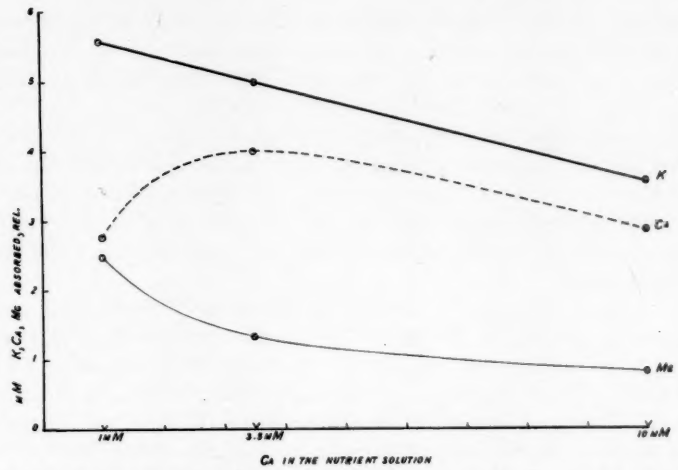


FIG. 7. Smoothed absorption curves of potassium, calcium, and magnesium with varying supply of calcium in the nutrient solution.

in the two experiments with varying Ca in the nutrient solution. With increasing Ca supply, K decreases per unit dry weight in a straight line curve. The curve for the variable Ca at first increases and later parallels the K curve. When it is recalled that total absorption of Ca parallels growth, it suggests that this element fills a function closely related to the growing processes, *e.g.*, cell division. Ca was stored in the tissues in proportion to

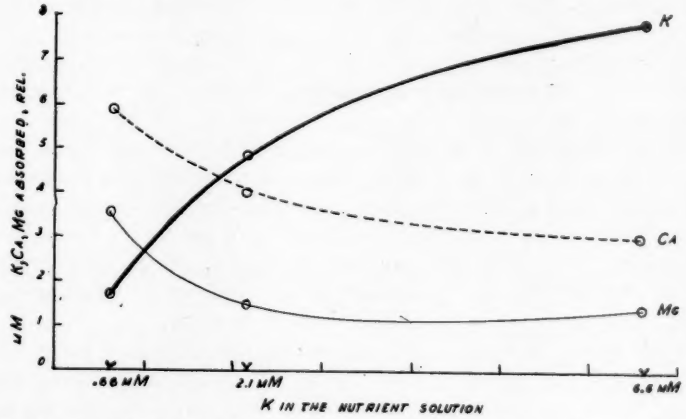


FIG. 8. Smoothed absorption curves of potassium, calcium, and magnesium with varying supply of potassium in the nutrient solution.

the tissues formed. In the absence or insufficient supply of Ca, cells at the heliotropic or geotropic growing points fail to develop.

Magnesium decreases in the plant as the Ca concentration is increased in the nutrient solution. The trend of the curve, however, seems to indicate that the greater the outer concentration of Ca, the less depressing is the effect on Mg; this is apparently a result of decreased inner concentration of Ca.

In figure 8 are shown the smoothed curves of absorption of K, Ca and Mg under varying concentrations of K in the nutrient solution. Potassium is absorbed somewhat in proportion to K present in the solution, while Ca and Mg are decreased.

Any study of base absorption and translocation in plants must consider antagonism. BURSTRÖM (**2**) in studying cation absorption in oats defines "antagonism" as the ability of one ion to retard the absorption of another. RUBINSTEIN (**13**) recognizes a "pseudo-antagonism" for cases of apparent antagonism due to difference in ion concentration. The writer believes that a true picture of antagonistic phenomena cannot be correctly estimated without considering both categories. It should also be considered what forces or conditions govern antagonism and pseudo-antagonism which must necessarily be operative in the root zone. In antagonism where the force or nature of one ion is matched with one of different character, it is evident that the permeability of the plasma membrane of the outer root cells is involved. LUNDEGÅRDH (**6**) has pointed out that this permeability to elements (ions) in solution stands in a direct relation to the diameter of the ions and to a certain extent to their specific mobility. Furthermore, the degree of hydrolization of inorganic ions is a factor, since they vary in their affinity to molecules of water. This affinity increases with valence and decreases with size of ions. Thus, K ions possess a greater mobility than Ca ions and the latter a greater mobility than Mg ions. It is concluded therefore that the cause of antagonism is due to variation in mobility and size of ions, and to a certain degree—hydrolization.

Pseudo-antagonism, evident in the end result in the same way as antagonism, operates through the force of mass action, and is not a matter of ion equivalence but of a difference in ion concentration. The operation of mass action thus produces an apparent antagonism, or more properly, a pseudo-antagonism. It is clear that both these categories, due to different causes, warrant consideration in a discussion of ion absorption. The curves presented in figures 7 and 8 support the views given above.

In the present work the evidence confirms the findings of other investigators (**9, 11**) that Mn is essential for normal growth and development of plants. The absorption of K, Ca, and Mg in the presence and absence of Mn has been discussed under the individual experiments. It may be of interest, however, to view the relative absorptions of K and Ca, *i.e.*, in percentages

of quantities supplied in the nutrient solutions with and without Mn. A graphical summary is given in figure 9.

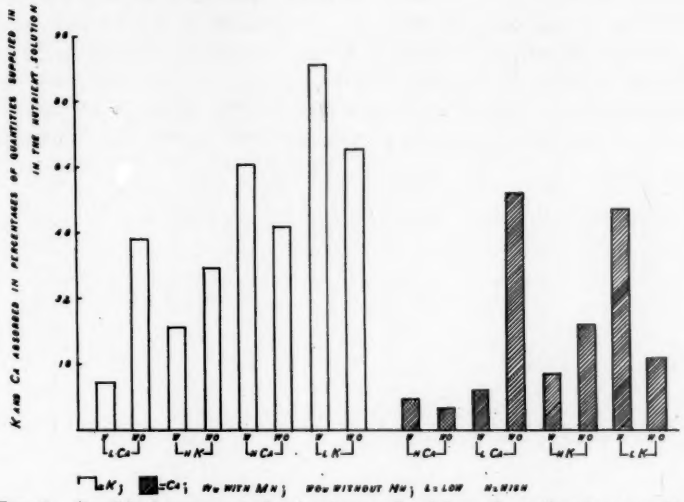


FIG. 9. Graphical summary of potassium and calcium absorption in percentages of quantities supplied in the nutrient solution, with and without addition of manganese.

The efficiency of K utilization was greater in absence of Mn at "low Ca" and "high K." The reverse is true at "high Ca" and "low K." Where Ca dominated, Mn facilitated the utilization of K, which was adversely affected when K dominated. The greatest relative utilization occurred at low-K supply in the presence of Mn.

In viewing Ca absorption, it is evident that where K is dominating ("low Ca," "high K"), utilization of Ca is improved in the absence of Mn. Where, however, Ca dominated ("high Ca," "low K") addition of Mn benefitted the utilization of Ca. The highest efficiency of Ca utilization was derived from a low-Ca supply without Mn. Therefore, it is concluded that Mn retards the absorption of Ca, when pseudo-antagonism is operative on the Ca ions.

From a practical viewpoint, Mn is of greater importance in soils where Ca dominates (a high-Ca level or a relatively low level of K) than where Ca is deficient. Applications of Ca (lime) should be guided by the actual content of active Mn in the soil (**4**). In Rhode Island (**3**) where soils are generally low in Mn, crops have shown symptoms of Mn deficiency through excessive liming.

On the other hand, in the tobacco growing district of Connecticut where soils are kept rather acid, the problem of excessive absorption of Mn is a serious one since a content of more than 0.03 per cent. Mn per unit dry

weight of the leaf causes discoloration of the ash, known to the cigar trade as muddy or brick colored ash (**1**).

The diagram in figure 10 shows the absorption of Ca in percentages of Ca supplied in presence and absence of B. Most striking is the treatment "low K." The lowest efficiency obtained in the utilization of Ca occurred in the absence of B while the highest occurred with boron present. Here Ca was dominating as in "high Ca," where B had little effect on Ca utilization. This may mean that B produces a pseudo-antagonism to Ca. Where this is

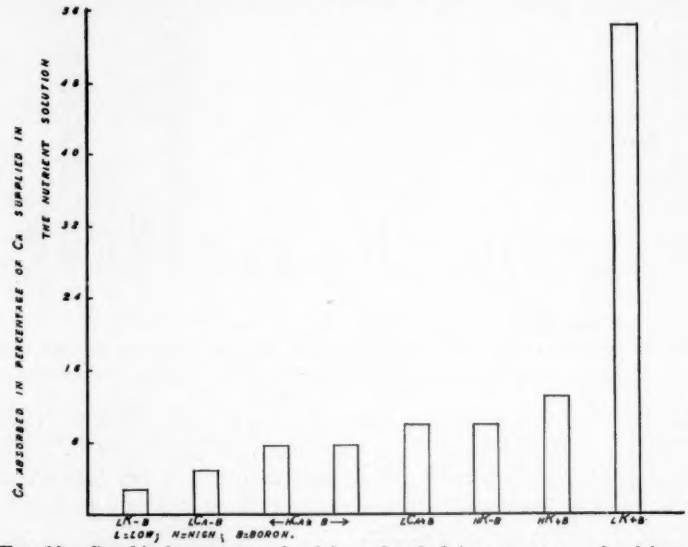


FIG. 10. Graphical summary of calcium absorbed in percentage of calcium added to the nutrient solution in the presence or absence of boron.

strong enough, addition of B does not improve the efficiency of Ca. This, however, should not be confused with the positive physiological need of B. Moreover, where Ca was physiologically deficient ("low Ca," "high K") the presence of B facilitated the utilization of Ca. It is concluded, therefore, that B aids the absorption of Ca. The practical aspect of this finding is the application of B to soils where Ca absorption needs to be improved, that is, where K, Mg, H, and NH_4 (**7**) or other ions exert antagonism or pseudo-antagonism sufficiently to limit Ca absorption below growth requirements. In line with this are the observations of MCMURTREY (**10**) on B deficiency symptoms of tobacco under field conditions.

There is some resemblance between Ca deficiency and B deficiency symptoms of tobacco in the injury to the terminal bud, although MCMURTREY (**8**) states that lack of boron affects the bases of the young leaves, while tops and margins are affected by lack of Ca. The writer has observed, however, that

die-back of the mid-vein at the base also occurs on Ca deficient tobacco plants upon longer standing. WARINGTON (**15**) has shown that a close association exists between Ca and B in the absorption and utilization of Ca. It is likely that a close association also prevails within the plant. From the work by JOHNSTON and DORE (**5**) it may be inferred that the production of pectic substances may be related to the presence of B. They state that B is apparently essential to cell division, to which function the presence of Ca-pectate is also related in the wall formation (**12**). Further studies are required to elucidate the definite relationship between Ca and B.

In this paper the expression "Translocation Quotient" (Tq) has been introduced. It expresses the ratio between ions present in stems and leaves (per unit dry weight) and identical ions found in the roots. It would appear that the use of a quotient or ratio might aid in any interpretation of data. The nearest approach to a "Tq" is BURSTRÖM'S (**2**) "total absorption per unit root weight," employed in the present work for computations involved in figures 2 and 4. While this latter calculation is useful to establish antagonism, simpler calculations shown in this paper have served a similar purpose and perhaps more convincingly. The "Tq" may be useful in studies of rate of growth where magnitude and variation in treatment are compared in order to observe cation as well as anion translocation.

Summary

In this paper work has been reported on absorption of bases (mainly the three cations K, Ca, Mg) studied by means of water cultures. Tobacco of a type commonly grown in the Connecticut Valley (Havana Seed) was used as a test plant. Moreover, the interrelated antagonistic effect of these ions were observed. A distinction between antagonism and pseudo-antagonism was made, and the reason for it outlined. It was shown that, while K is antagonistic to Ca, this ion under certain conditions may effect a pseudo-antagonism toward K. The same is true with Ca versus Mg.

The effect of varying quantities of K and Ca in the nutrient solution on the absorption of Fe, Na and P was also observed. Ca depresses the uptake and translocation of Fe and is antagonistic to Na. The translocation of P is aided by Ca.

The absorption of the three cations was also studied in the absence and presence of B and Mn in the nutrient solutions. In general it was found that B aids absorption and utilization of Ca.

Mn under certain conditions has a regulating influence on the absorption of Ca, while this ion *per se* is antagonistic to Mn.

A "translocation quotient" (Tq) has been suggested in this paper to be used in connection with absorption data in order to facilitate interpretation of translocation phenomena.

This work was conducted in the laboratories of Professor H. LUNDE-

GÅRDH, Centralanstalten, Stockholm, Sweden. The author is greatly indebted to Professor LUNDEGÅRDH and his associate, Dr. HANS BURSTRÖM, for use of laboratory and equipment as well as valuable suggestions pertaining to this study.

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TRANSPIRATION AND PHYSICO-CHEMICAL PROPERTIES OF LEAVES AS RELATED TO DROUGHT RESISTANCE IN LOBLOLLY PINE AND SHORTLEAF PINE[1]

C. S. SCHOPMEYER

(WITH TWO FIGURES)

Introduction

In the reforestation program now being carried on by various state and federal agencies, many millions of tree seedlings are planted each year. An extremely large number of these planted seedlings are killed within the first year or two after planting. One of the major causes of the fatalities is the inability of seedlings to resist drought. If the properties involved in drought resistance of seedlings were known, and if those species and age classes of planting stock possessing such properties could be identified or developed, much better survival could be obtained in plantations of tree seedlings.

Drought resistance, the ability of a plant to endure permanent wilting without harm to subsequent development (**11**), has been induced in plants by repeated wilting and also by growing them in soil with a moisture content barely above the wilting percentage (**27, 28**). Observations on permanently wilted herbaceous plants (**27**) show that their transpiration rate may be less than one-fourth of the rate for turgid plants. Most conifers, however, do not show any external evidence of a wilted condition. Hence, in hardening conifers against drought, the wilting percentage of soil moisture must be previously determined to facilitate control of soil moisture conditions.

Although attempts have been made to correlate drought resistance with rate of transpiration, there is much evidence (**1, 2, 4, 21, 24, 32**) to support the statement of MAXIMOV and KRASNOSSELSKY-MAXIMOV (**12**) that the rate of transpiration when soil moisture is abundant cannot be used for judging drought resistance. Transpiration may be greatly reduced when soil moisture approaches the wilting percentage (**15, 19**) and the reduction may vary among plants of different morphological structure, but there appears to be no consistent relationship between drought resistance and rate of transpiration at soil moisture contents near the wilting percentage.

There is evidence of a correlation between drought resistance and bound water content of leaf tissue. KORSTIAN (**9**) found some indication of such a correlation in a preliminary study of southeastern tree species. The work of NEWTON and MARTIN (**17**) is particularly outstanding in that a very definite correlation was obtained in cereal crops and grasses between the degree of drought resistance and the bound water content. NOVIKOV (**18**) also

[1] This investigation was supported by a Fellowship in Forestry in the Graduate School of Arts and Sciences of Duke University.

found that the amount of bound water present characterized the degree of drought resistance in varieties of wheat when they were in a wilted state but not in a turgid state. He attributed an observed increase in bound water during hardening not to a decrease in total water but to physico-chemical changes in the cell contents.

Information on the relationship of osmotic pressure of cell sap to drought resistance is very conflicting. DRABBLE and DRABBLE (3) and LIVINGSTON (10) have shown that a high osmotic pressure cannot lower the vapor pressure of cell sap sufficiently to reduce water loss by transpiration to an appreciable extent. KORSTIAN (8) obtained evidence indicating that water absorption by plants depends on osmotic pressure, but STODDART (25) believes that a high osmotic pressure is a result of drought and not an adaptation to it. NEWTON and MARTIN (17) found that osmotic pressure was not a consistently reliable index of drought resistance. In comparing osmotic pressures of two or more species it is difficult to make a proper interpretation because in most cases it is not known whether differences are caused by different water contents or by different solute concentrations.

The primary purpose of this investigation was to ascertain whether the relative drought resistance of seedlings of two species can be determined by physico-chemical means. A secondary purpose was to obtain information on the behavior of seedlings during and after drought by a study of changes in physico-chemical properties. The approach was made by a study of transpiration, bound-water content, total water content, osmotic pressure, and calculated solute concentration in leaves of two species growing in a greenhouse under controlled soil moisture conditions. Loblolly pine (*Pinus taeda* L.) and shortleaf pine (*P. echinata* Mill.) were selected for comparison because both species occur within the same region with loblolly pine on mesic sites, and shortleaf pine on more xeric sites. The apparent adaptations of these species to different sites indicate that shortleaf pine is the more drought resistant of the two species.

Materials and methods

SEEDLINGS AND SOIL

A group of vigorous one-year-old seedlings of loblolly pine and of shortleaf pine were selected at the North Carolina State Nursery and moved to a greenhouse in December, 1935. The seedlings were planted in two-gallon buckets containing Congaree silt loam, the moisture constants of which are presented in table I. A watering tube extended down the side of each bucket into a layer of crushed rock in the bottom. The buckets were covered with a double layer of oilcloth to prevent evaporation of water from the soil. Soil moisture was maintained at 30 per cent. for a month after the completion of planting to permit the seedlings to become established.

TABLE I

MOISTURE CONSTANTS FOR CONGAREE SILT LOAM

ITEM	MOISTURE CONTENT (PERCENTAGE OF DRY WEIGHT)
	%
Moisture equivalent	27.3
Wilting coefficient	14.8
Wilting percentage	7.7
Saturation percentage	53.3

DETERMINATION OF TRANSPIRATION

The gravimetric method for the determination of transpiration was selected for this work. The buckets were weighed daily to determine the weight of water lost by transpiration which was expressed in grams per gram of oven-dried leaf tissue per day.

On April 4 the seedlings of each species were divided into three groups for a study of transpiration under three conditions of soil moisture. In the first group of seedlings, the soil moisture was brought to 30 per cent. at weekly intervals. This moisture content is believed to be approximately an optimum for growth. Between waterings the soil moisture in this group rarely went below 25 per cent. In the second group, the seedlings were left unwatered, and the soil moisture was gradually decreased to the wilting coefficient. Approximately six weeks elapsed before the wilting coefficient was reached. This treatment, according to TUMANOV (**27**), should induce drought hardening. In the third group, the soil moisture was allowed to reach the wilting coefficient, as in the second group, and was then brought back to 30 per cent. moisture. The latter treatment enabled the seedlings to recover from the effects of drought and to resume growth. In the first two groups, transpiration determinations were made at weekly intervals. In addition, daily determinations were made near the end of the experiments for all three groups.

DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES

After completing the transpiration determinations ten samples were collected from each species in each of the three soil moisture categories. Collections were made at 11 P.M. One sample consisted of all the leaves from one seedling. The fresh weight of the sample was obtained after placing it in a closed tin container. Each sample was macerated in a meat grinder and divided into three parts for the determination of bound water, total water, and freezing point, respectively.

The calorimetric method for the determination of bound water (**5, 13, 16, 20, 22, 23, 26**) was selected for this work. In making the determinations, ten

samples from each category were run through the procedure together. The samples, each consisting of about 8 grams of the macerated leaf tissue, were placed in tinfoil cups similar to those used by ROBINSON (**20**) and GREATHOUSE (**5**). The cups were placed in glass vials, tightly stoppered, and weighed to ± 0.001 gram. After weighing, the tinfoil cups containing the samples were placed in stoppered freezing tubes with two samples in each tube. The freezing tubes were suspended in a freezing bath at a temperature of – 20° C. ± 2° C. through holes in a cover over the bath. The samples were left in the bath for approximately 12 hours. A pint thermos bottle, containing 250.0 gm. of water was used for a calorimeter. The initial temperature of the water in the calorimeter was determined by using a mercury thermometer which was read to ± 0.005° C. A sample, enclosed in its tinfoil container, was transferred from the freezing bath to the calorimeter. After continuous stirring with a motor-driven stirring rod for approximately 8 minutes, thermal equilibrium was reached and the final temperature was recorded. A correction factor for the heat absorbed by the calorimeter and accessories was obtained by placing 8 ml. of water in the tinfoil cups and following the procedure just outlined. The formulae used in calculating the bound water contents are essentially the same as those used by GREATHOUSE (**5**).

In preparing samples for specific heat determinations on the dry matter in the leaf tissue, leaves were dried for 24 hours at 95° C. and then ground to a fine dust with a mortar and pestle. The ground leaf tissue was placed in tinfoil cups and dried to constant weight ± 0.001 gram at 95° C. The specific heats were determined using the same procedure as for bound water except that benzene was used instead of water in the calorimeter. Benzene, having a lower specific heat, facilitated measurement of the temperature change in the calorimeter. The specific heats of benzene and of tin were obtained by plotting their values as given in the *International Critical Tables* (**31**) and then interpolating for the desired temperature. The specific heats of water and of ice were obtained from the *Handbook of Chemistry and Physics* (**7**). For loblolly pine the mean of 5 specific heat determinations on the dry leaf tissue was 0.213 with a standard error of ± 0.002; the mean of 5 determinations on shortleaf pine was 0.208 with a standard error of ± 0.006.

The total water content of a sample was assumed to be the difference between the fresh weight and the weight after drying the macerated tissue to constant weight ± 0.001 gm. at 95° C. The water content was calculated on a dry weight basis.

Freezing point determinations were made on the leaf tissue using the thermo-electric method (**14**). The apparatus consisted of two thermocouples connected in series to a galvanometer. One junction was maintained at 0° C. The other junction was sealed into the tip of a hypodermic needle

and inserted into a 1.5-cm. length of glass tubing containing a portion of the macerated leaf tissue. The junction with the enclosing sample was placed in a freezing tube suspended in a freezing bath. The amount of undercooling and the observed freezing point were recorded. The true freezing point and the osmotic pressure were calculated from the tables of HARRIS and GORTNER (6).

Since osmotic pressure of a tissue varies with total water content, the usefulness of osmotic pressure for comparisons of solute concentration in tissues of different water contents is very limited. In an attempt to obtain a more stable factor for such comparisons, the following conversion was devised.

$$\frac{\pi \times [H_2O]}{22.4} = [\text{Solute}]$$

where

π = osmotic pressure
$[H_2O]$ = grams of water per gram of dry leaf tissue
[Solute] = number of gram molecular weights (mols) of solute per kilogram of dry leaf tissue
22.4 = the osmotic pressure of a mol of undissociated solute in 1000 grams of water

This conversion does not give a true measure of solute concentration because the relative amounts of ionized and un-ionized solutes are not known. This formula merely converts osmotic pressure to its equivalent in mols of undissociated solute on a dry weight basis and thus gives a factor which probably does not vary to a great extent with changes in water content. This factor will be designated as *solute concentration* in the following discussion, but

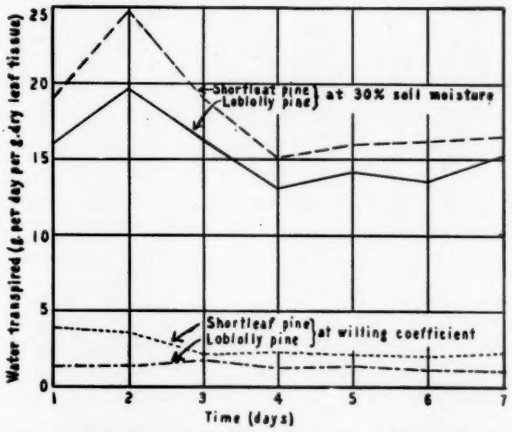

FIG. 1. Daily transpiration of loblolly pine and shortleaf pine at 30 per cent. soil moisture and at the wilting coefficient. Each point is the mean value of 9 determinations.

actually it is just a rough approximation to the true solute concentration because of the unknown degree of dissociation.

Results and discussion

Transpiration

Shortleaf pine had a higher daily rate of transpiration than loblolly pine with soil moisture at 30 per cent. and also at the wilting coefficient. Mean values of nine trees of each species in the two soil moisture categories on seven successive days are shown in figure 1. The differences between the two species are highly significant at both soil moisture contents as shown by the analysis of variance in table II. Significant also is the variation in

TABLE II

ANALYSIS OF VARIANCE IN TRANSPIRATION RATES OF LOBLOLLY PINE AND SHORTLEAF PINE

Source of variation	Degrees of freedom	Sum of squares	Mean square	Calculated *F* value
Soil moisture at 30 per cent.				
Species	1	222.60	222.60	59.52†
Days	6	915.56	152.59	40.80†
Interaction	6	45.30	7.55	2.02*
Error	112	419.35	3.74	
Total	125	1602.81		
Soil moisture at wilting coefficient (14.8 per cent.)				
Species	1	41.18	41.18	76.26†
Days	6	16.99	2.83	5.24†
Interaction	6	16.06	2.68	4.96†
Error	112	60.27	0.54	
Total	125	134.50		

* Not significant (less than 5 per cent. point).
† Highly significant (greater than 1 per cent. point).

transpiration rates resulting from the variation caused by differences in temperature and humidity from day to day. These results indicate that the apparently greater ability of shortleaf pine to resist drought cannot be attributed to an ability to conserve water by retarding transpiration. This conclusion agrees with the statement of MAXIMOV (**11**) that xeric plants are distinguished by a higher rate of transpiration than that of more mesic plants.

The rate of transpiration of loblolly pine gradually diminished over a period of six weeks as the decreasing soil moisture approached and passed the wilting coefficient (14.8 per cent. moisture). At the end of the six-week period, the rate was only 15.8 per cent. of that at 30 per cent. soil moisture. This gradual reduction is not to be expected according to the results of VIEHMEYER and HENDRICKSON (**30**). They found that extraction of water

from soil by peach trees is not affected by soil moisture content until it is reduced to the wilting percentage. Hence transpiration would be expected to remain constant until the wilting percentage is reached. The gradual reduction observed in this work can be explained, however, by assuming that at the beginning of the period of drought, only a portion of the roots were in soil at the wilting coefficient; and as more and more of the roots extracted all of the water available to them, transpiration was gradually reduced. The transpiration rates of the two species at the wilting coefficient, shown in figure 1, are not as low as the negligible amounts of water lost by western conifers as observed by PEARSON (19). The calculated wilting coefficient for the soil used in this study, however, as shown in table I, is approximately 7 per cent. higher than the true wilting percentage as determined experimentally. Hence there may have been an appreciable amount of available soil moisture at the calculated wilting coefficient.

With soil moisture at 30 per cent. after having been reduced to the wilting coefficient, differences in transpiration rates between the two species were not significant. A comparison of the transpiration rates of loblolly

TABLE III

ANALYSIS OF VARIANCE IN BOUND WATER, TOTAL WATER, OSMOTIC PRESSURE, AND SOLUTE CONCENTRATION IN LEAF SAMPLES OF LOBLOLLY PINE AND SHORTLEAF PINE COLLECTED AT 11 P. M.

ITEM	SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	CALCULATED *F* VALUE
Bound water	Species	1	109.54	109.54	1.65*
	Soil moisture	2	1274.29	637.65	9.61†
	Interaction	2	129.87	64.94	0.98*
	Error	54	3581.43	66.32	
	Total	59	5095.13		
Total water	Species	1	1920.6	1920.6	10.55†
	Soil moisture	2	24202.3	12101.2	66.49†
	Interaction	2	187.1	93.6	0.51*
	Error	54	9827.2	182.0	
	Total	59	36137.2		
Osmotic pressure	Species	1	66.03	66.03	51.19†
	Soil moisture	2	540.34	270.17	209.43†
	Interaction	2	129.06	64.53	50.02†
	Error	54	69.57	1.29	
	Total	59	805.00		
Solute concentration	Species	1	1.3735	1.3735	88.61†
	Soil moisture	2	3.1324	1.5662	101.05†
	Interaction	2	0.6717	0.3358	21.66†
	Error	54	0.8373	0.0155	
	Soil moisture	2	24203.0	12101.0	66.49†
	Total	59	6.0149		

* Not significant.
† Highly significant.

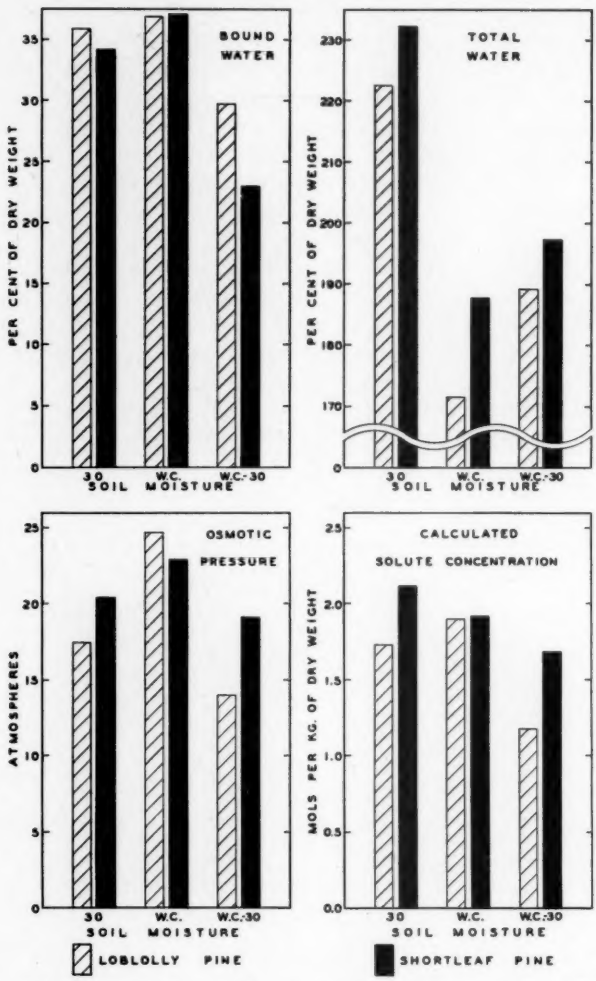

FIG. 2. Mean values of bound water, total water, osmotic pressure, and solute concentration in leaves of loblolly pine and shortleaf pine under various soil moisture conditions. Each value is the mean of 10 determinations.

30 = Soil moisture maintained continuously at 30 per cent.

W.C. = Soil moisture at the wilting coefficient.

W.C.–30 = Soil moisture at 30 per cent. after having been reduced to the wilting coefficient.

TABLE IV

SIGNIFICANCE OF DIFFERENCES BETWEEN MEANS OF PHYSICO-CHEMICAL PROPERTIES OF LEAVES OF LOBLOLLY PINE AND SHORTLEAF PINE

CATEGORIES COMPARED*	BOUND WATER		TOTAL WATER		OSMOTIC PRESSURE		SOLUTE CONCENTRATION	
	DIFFERENCE BETWEEN MEANS	*t* VALUE†	DIFFERENCE BETWEEN MEANS	*t* VALUE†	DIFFERENCE BETWEEN MEANS	*t* VALUE†	DIFFERENCE BETWEEN MEANS	*t* VALUE†
	%		%		*Atm.*		*Mols.*	
SP:30–LP:30	−1.76	0.5	9.66	1.5	2.98	5.8	0.384	8.3
SP:WC–LP:WC	0.36	0.1	16.23	2.7	−1.85	3.4	0.016	0.3
SP:WC:30–LP:WC:30	−6.71	1.8	8.06	1.3	5.17	10.1	0.512	11.1
LP:30–LP:WC	−1.09	0.3	51.02	8.5	−7.26	14.2	−0.168	3.6
LP:30–LP:WC:30	6.06	1.7	33.37	5.5	3.46	6.8	0.556	12.1
LP:WC–LP:WC:30	7.15	2.0	−17.65	2.9	10.72	21.0	0.724	15.7
SP:30–SP:WC	−3.01	0.8	44.45	7.4	−2.42	4.8	0.200	4.3
SP:30–SP:WC:30	11.16	3.1	34.97	5.8	1.28	2.5	0.428	9.3
SP:WC–SP:WC:30	14.13	3.9	−9.47	1.5	3.70	7.3	0.229	5.0

* SP = shortleaf pine. LP = loblolly pine.
30 = samples from trees in soil at 30 per cent. moisture.
WC = samples from trees in soil with moisture content at the wilting coefficient.
WC:30 = samples from trees in soil at 30 per cent. moisture after having been reduced to the wilting coefficient.

† $t = \frac{\text{difference between means}}{\text{standard error of difference}}$. Values of t greater than 2.0 are considered significant.

pine supplied continuously with 30 per cent. soil moisture and of the same species at 30 per cent. soil moisture after having been reduced to the wilting coefficient showed no significant difference. No comparison was made on the transpiration rates of seedlings of shortleaf pine at these two soil moisture contents.

Physico-chemical properties of leaf tissues

Analyses of variance were made on all physico-chemical data and are presented in table III. These analyses show that total water, osmotic pressure, and solute concentration in leaves of shortleaf pine and loblolly pine differed significantly between species as well as between soil moisture categories. The analyses also show that soil moisture treatments caused significant differences in bound water contents, and that species did not. The mean values of each physico-chemical property in each category are presented graphically in figure 2. Tests of the significance of differences between individual means are presented in table IV.

The bound water content values may be subject to criticism because of the large variation within categories as indicated by the magnitude of the mean square of error in the analysis of variance in table III. Two factors may contribute to this variation: first, inconsistencies in the technique used in making the determinations; and second, variation in the amount of bound water from tree to tree within categories. No replicated bound water determinations were made on substances known to be homogeneous; hence an absolute check on the reliability of the method is not available. The replications of the specific heat determinations, however, and the calorimeter factor determinations, presented in table V, are very consistent. Since these determinations were made using the same apparatus that was used in making the bound water determinations, the consistency of the specific heat values and the calorimeter factors is an indication of the reliability of the technique.

TABLE V

Comparison of variation in replicated determinations of bound water, specific heat, and the calorimeter factor

Item	Specific heat of loblolly pine	Calorimeter factor	Bound water*
Number of replications	5	4	10
Mean	0.213	1.070	35.81
Standard deviation	0.00336	0.00895	7.78
Standard error	0.00151	0.00448	2.46
Standard error expressed as a percentage of the mean	0.7	0.4	6.9

* Determinations were made on leaves of loblolly pine when soil moisture was at 30 per cent.

When soil moisture was at 30 per cent., leaves of shortleaf pine were higher in total water, osmotic pressure, and solute concentration than were leaves of loblolly pine. Bound water contents were not significantly different in leaves of the two species. Consideration of these data alone might lead to the conclusion that the greater drought resistance of shortleaf pine is attributable to osmotic effects. Data on the physico-chemical properties under the other two soil moisture conditions show, however, that osmotic effects have no direct connection with the relative drought resistance of loblolly pine and shortleaf pine.

Changes in the seedlings which occurred when soil moisture was reduced from 30 per cent. to the wilting coefficient furnish some interesting information on the behavior of the two species during drought hardening. Seedlings of both species became dormant as judged by the cessation of growth and the setting of terminal buds. In leaves of both species osmotic pressure increased, and total water decreased considerably. Solute concentration apparently decreased in leaves of shortleaf pine, but increased in leaves of loblolly pine. These changes in solute concentration, although small, are statistically significant. They are not believed to be physiologically significant, however, because of the fact that the changes are in opposite directions in the two species.

No significant change occurred in the bound water content of the leaves of either species when soil moisture was reduced from 30 per cent. to the wilting coefficient. This fact leads to the assumption that no appreciable change took place in the amount of water-binding colloids and crystalloids in leaves of the two species during the drought hardening period. The fact that no consistent change in solute concentration occurred in the two species when soil moisture was reduced may be regarded as evidence partially supporting this assumption.

Physico-chemical differences between species when soil moisture was at the wilting coefficient show which properties cannot be used for determining the relative drought resistance of the two species and also show which factors might be indicative of the relative drought resistance. With soil moisture at the wilting coefficient, leaves of shortleaf pine had a higher total water content and a lower osmotic pressure than leaves of loblolly pine. Solute concentration and bound water contents were practically the same in both species.

Since solute concentration on a dry weight basis was the same in the two species when soil moisture was at the wilting coefficient, it follows that the observed difference in osmotic pressure was the result of the difference in total water content. Since osmotic pressure was influenced in this way, it cannot be used as an indicator of the relative drought resistance of the two species.

The simultaneous occurrence of a higher total water content and a higher transpiration rate in leaves of shortleaf pine when soil moisture was at the wilting coefficient, indicates that this species had a faster rate of absorption of water from soil than loblolly pine when soil moisture was limited. This ability of shortleaf pine might be attributed to a lower ratio of evaporating leaf surface to absorbing root surface. A superficial examination of the root systems of the seedlings used in this work indicated that shortleaf pine seedlings had a lower top-root ratio than loblolly pine. More exact measurements are necessary, however, to prove the point.

The ability of a seedling to maintain a higher total water content in its leaves when soil moisture is limited may be an aid to survival during periods of drought in that it might prolong the period of permanent wilting and thus delay the occurrence of a water content in the leaves below the minimum necessary for life. Such a prolongation of the period of permanent wilting, however, would enable a seedling to survive periods of drought only as long as the soil moisture content did not go below the wilting percentage for a prolonged period.

The fact that shortleaf pine, the more drought resistant of the two species studied, had the greater total water content in its leaves when soil moisture was at the wilting coefficient, leads to the conclusion that the magnitude of the total water content may be used as an indicator of the relative drought resistance of the two species under the conditions of this experiment.

In view of the correlation obtained by other workers between bound water contents and relative drought resistance, an explanation of the lack of such correlation in this study, when soil moisture was at the wilting coefficient, is pertinent. NEWTON and MARTIN (**17**) who obtained a good correlation between drought resistance and bound water contents of cereal crops and grasses, used the cryoscopic method for the determination of bound water in expressed sap. With this method, only the amount of bound water held by the colloids, ions, and molecules expressed in the sap is determined. MEYER (**13**) stated that a considerable amount of water may be bound in cell walls of plant tissues. Since leaves of many species of the genus Pinus, including loblolly pine and shortleaf pine, are characterized by a hypodermal layer of cells with thick walls, the amount of water bound by dispersed substances within the cells may be negligible compared to the amount of water bound in the cell walls. In this investigation, bound water determinations were made on macerated leaf tissue which included both cell walls and cell contents. The amount of water-binding surface in cell walls per unit weight of dry leaf tissue may have been sufficiently uniform in the two species to prevent the detection of small differences in the amount of bound water in the cell sap. Since no data are available on the relative amounts of bound water in cell walls and in cell sap, any interpretation of

the bound water contents of the leaf tissue must be based on their face value. Hence, the magnitude of the bound water content of the leaf tissue as determined in this investigation, is not a factor which makes shortleaf pine more drought resistant than loblolly pine.

Striking changes occurred in the leaves of the two species after soil moisture was increased from the wilting coefficient to 30 per cent. The physico-chemical properties were determined one week after the soil moisture had been brought back to 30 per cent. During that week dormancy was broken and growth was resumed. Total water contents of leaves were greater than when soil moisture was at the wilting coefficient, but less than when seedlings were first supplied with 30 per cent. soil moisture. Large decreases in bound water, osmotic pressure, and solute concentration occurred in leaves of both species. Each of these three properties were lower in magnitude than in either of the other two soil moisture categories. These results agree with those of VASSILIEV and VASSILIEV (**29**) who found that wheat plants grown in soil with insufficient water and then watered abundantly were lower in water content as well as in monosaccharides and sucrose than check plants grown continuously with abundant soil moisture.

Total water, osmotic pressure, and solute concentration were higher in leaves of shortleaf pine than in leaves of loblolly pine after 30 per cent. soil moisture was restored. No significant difference in the bound water contents of the two species was observed in this soil moisture category.

The decreased solute concentration and the decreased bound water contents in the leaves of the two species indicate a rapid depletion of food reserves, including water-binding colloids, in the leaves when dormancy resulting from drought is broken by the restoration of abundant soil moisture. The fact that the decrease in solute concentration was not as great in shortleaf pine as in loblolly pine indicates that the former species may have maintained a better balance between utilization and synthesis of soluble food reserves. This closer balance may enable shortleaf pine to recover more rapidly from the effects of drought, and hence it may be a factor contributing to the ability of this species to survive on drier sites than those on which loblolly pine occurs.

Summary

Determinations of transpiration, bound water, total water, and osmotic pressure were made on seedlings of loblolly pine and shortleaf pine to obtain information on the behavior of these species during and after drought and to ascertain whether any of these factors can be used as indicators of relative drought resistance. Solute concentration, calculated from data on osmotic pressure and total water content, was a valuable aid in interpreting the osmotic pressure differences.

With soil moisture at 30 per cent., shortleaf pine had a higher transpira-

tion rate, more total water, a higher osmotic pressure, and a greater solute concentration than loblolly pine. Bound water contents were not significantly different in the two species.

With soil moisture at the wilting coefficient, shortleaf pine had a higher transpiration rate, more total water, and a lower osmotic pressure than loblolly pine. Solute concentration and bound water contents were practically the same in both species.

When soil moisture was restored to 30 per cent. after having been reduced to the wilting coefficient, leaves of shortleaf pine had more total water, a greater osmotic pressure and a higher solute concentration than leaves of loblolly pine. Transpiration rates and bound water contents were practically the same in both species.

Bound water contents decreased in both species when soil moisture was restored to 30 per cent. after having been reduced to the wilting coefficient.

Points observed in this study which may contribute to the greater drought resistance of shortleaf pine compared to that of loblolly pine are as follows:

1. Shortleaf pine absorbed more water from the soil and at the same time maintained a higher total water content in its leaves even when soil moisture was limited.
2. Shortleaf pine maintained a higher solute concentration when recovering from the effects of drought; that is, when 30 per cent. soil moisture was restored.

The data also show that the greater drought resistance of shortleaf pine cannot be attributed to an ability to conserve water either by retarding transpiration or by forming bound water. Further, the greater drought resistance of this species cannot be attributed to a higher osmotic pressure.

NORTHERN ROCKY MOUNTAIN FOREST
AND RANGE EXPERIMENT STATION
MISSOULA, MONTANA

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SOME INVESTIGATIONS ON THE ASSIMILATION OF APPLE LEAVES

J. G. WAUGH

(WITH FIVE FIGURES)

Introduction

The assimilation of apple leaves subjected to various environmental conditions has been studied by a number of investigators. Extensive investigations have been made by HEINICKE and HOFFMAN (**10**) for individual leaves under natural conditions and more recently by HEINICKE and CHILDERS (**9**) for the foliage of an entire tree. These investigations, however, involved determinations of the average rate of assimilation over periods of several hours. This study was originated to obtain information on the assimilation of individual apple leaves under varying conditions over more brief periods of time than used heretofore. In the following experiments apple leaves were subjected to varying conditions in the laboratory and determinations of the rate of assimilation made in hourly periods.

Materials and methods

APPARATUS

MEASUREMENT OF CARBON DIOXIDE.—Assimilation was estimated by determining the differences in carbon dioxide content between a continuous stream of normal air and a similar stream of air that was passed over leaf tissue confined in a special assimilation chamber. The carbon dioxide of the air was determined with a maximum error of 2 per cent. and an average error of approximately 1 per cent. of the atmospheric concentration by an apparatus described by WAUGH (**22**).

OBTAINING AIR SAMPLES.—Since the experiments were performed in the laboratory where the carbon dioxide content of the air fluctuated violently and was usually considerably higher than that of the normal atmosphere, it seemed desirable to use fresh air from the outside for the experiments. In figure 1 is illustrated the apparatus for obtaining the air samples. *M* represents a leaf on which the experiments are made and *K* an air chamber for drawing air over the leaf. The inlet of the air chamber is fitted with a tube, *T*, which leads to outside air. A suction pump is attached at *J* for drawing air over the leaf and the rate of air flow is determined and adjusted by means of a Sargent wet-test gas meter, *I*, which is accurate to 28 ml. The leaf is subjected to the appropriate external conditions, side tube *E*, on T-tube *G* is shut off, and the three-way stopcock turned so that air is drawn through the air-chamber, *K*, through *L*, through *G* and *H* to the gas meter, *I*, and out at *J*.

The method of taking samples was as follows: A sampling bottle, *A*, was clamped in an inverted position in the ringstand, *F*. The side tube, *E*, was attached to the long tube of the sampling bottle and a small tube, *C*, drawn out to a jet was attached to the short tube of the sampling bottle. This small tube was adjusted to allow the water to run out at an appropriate rate, and consequently determined the rate of flow of air into the sampling bottle. Tubes were made which gave rates of air flow from 5 to 80 liters per hour; where more accurate data were required, stopwatch readings were taken. In

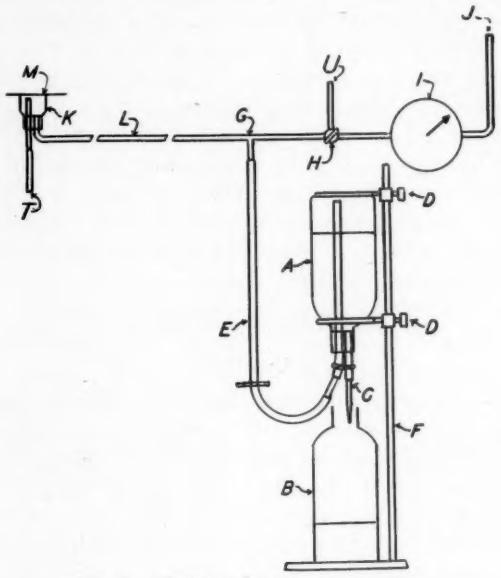

FIG. 1. Method of taking air samples.

some cases when the sample was taken, the suction was shut off from the cup chamber by turning *H*, and the air flowed over the leaf directly to the sampling bottle. In other cases, suction was applied and a sample taken slowly over a long interval of time with a high rate of air flow over the leaf. In fact, a considerable number of variations were obtained and records were kept of the temperature, barometric pressure, and other factors which might have their influence on the data. The method of taking the air check was very simple and consisted in attaching a sampling bottle to a tube which led air in directly from the outside. The carbon dioxide in the air samples was determined by the apparatus described by WAUGH (**22**).

AIR CHAMBER.—In the apple leaf the stomates are confined to the lower side and in figure 2 is given a diagram of an air chamber used in these experi-

ments which is a modification of the original chamber used by HEINICKE (6). The chamber *D* is made by cutting off an 8-oz. cylindrical bottle approximately 6 cm. in diameter about 3 cm. from the shoulder. The cut edge is ground flat and fitted with a rubber cushion *C* covered with soft grafting wax. A 3-hole no. 7 rubber stopper *G* provided with two 6-mm. inside diameter glass tubes, *H* and *I*, and a thermocouple *F* (to be described later), is fitted into the neck of the bottle. A brass ring *A* which holds the leaf *B* against the cushion *C*, is held in position by two rubber bands *EE*. The ring is coated with aluminum paint to reflect light and avoid excessive heating of the metal. It has a felt gasket *J* underneath to prevent injury to the leaf by pressure. The chamber is sturdy and may be clamped rigidly in any suitable position.

Two sizes of air chambers providing effective assimilating leaf areas of 13.18 and 19.64 cm.^{2} were used in these experiments. In fitting the leaf to an air chamber, it was carefully pressed down without injuring it by means of the brass ring *A* on the wax-covered rubber cushion, and the wax adapted itself to the veins and contours of the leaf forming a seal which was

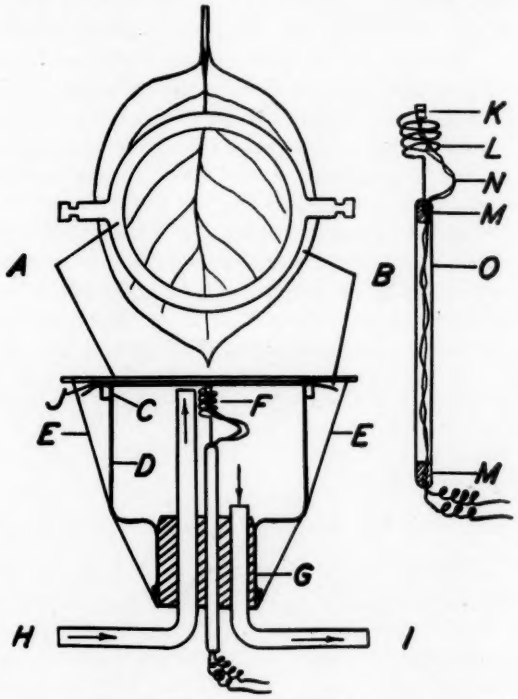

FIG. 2. Air chamber.

very nearly air-tight. Tests indicated that when a healthy apple leaf free from imperfections was used with a rate of air flow of 60 liters per hour through the air chamber, the pressure differential exerted on the leaf was small and the leakage negligible. During the periods when experiments were not being performed, the leaf was removed from the air chamber. The leaf was constantly checked for imperfections and perforations. In re-fitting the leaf to the air chamber, care was taken to make the leaf coincide with the impressions in the wax to obtain a good seal and also that the same portion of the leaf as before came under observation for comparable data.

MEASUREMENT OF LEAF TEMPERATURES.—Leaf temperatures were measured with a thermocouple system *F* which is drawn to enlarged scale to the right of the air chamber in figure 2. The thermocouple support consists of a 3-mm. outside diameter glass tube *O* approximately 7.5 cm. long. A light phosphor-bronze wire is shaped into a spiral *L* which holds a small cylinder of cork *K*. On the top of this cork cylinder is supported a copper-constantan thermocouple which lies flat on the surface. The thermocouple leads *N* are brought through the tube *O* and air-tight seals made with sealing wax at *MM*. The thermocouple system was installed in the air chamber so that the thermocouple pressed lightly against the under surface of the leaf near the midrib without injuring it. The thermocouple with its supporting cork cylinder was less than 3 mm. in diameter so that very little of the leaf surface was involved. The reference thermocouple was immersed in the thermostatic water bath of the carbon dioxide measuring apparatus, which was held at 30° ± 0.01° C. Leaf temperatures were measured accurate to 1° C.

METHOD OF APPLYING AND MEASURING LIGHT.—Carbon-dioxide assimilation is expressed on the basis of the milligrams of carbon dioxide absorbed per 100 cm.^{2} of leaf surface per hour. Light intensity is expressed in gram calories per cm.^{2} of leaf surface per hour, and the milligrams of carbon dioxide absorbed per gram calorie of light may be obtained by the following relation:

$$\text{mg. } CO_2 \text{ assimilated per gm. cal. of light} = \frac{\text{mg. } CO_2 \text{ assim. per 100 cm.}^2 \text{ per hour}}{100 \times \text{gm. cal. light per cm.}^2 \text{ per hour}}$$

The light intensity may be expressed approximately in foot-candles by the following relationship (**12**):

$$\text{Light intensity in foot-candles} = 115 \times \text{gm. cal. per cm.}^2 \text{ per hour}$$

A Mazda 150-watt, 120-volt clear glass lamp with 60 cycles A.C. per second was used to supply light to the leaves. In some experiments, it was fitted with a white enamelled reflector. The light intensity applied to the leaf was varied by placing the light at various distances from the leaf and vertical to its surface. An Eppley pyrheliometer (**13**) was used to determine the light intensity, and calibration curves giving the relation between the dis-

tance of the light from the leaf and the intensity of the light falling on the leaf were constructed. At close range where there was danger of burning the leaf owing to excessive heating, an electric fan was used to cool the leaf. This was placed about 60 cm. from the leaf so that it did not interfere with the light, and the air was driven horizontally across the surface of the leaf.

RECORDS.—Records were kept of the air temperature, leaf temperature, barometric pressure, light intensity, leaf area, rate of air flow, condition of leaf under observation, general condition of the trees and any other factors which might have their influence on the data. Since the temperature of the laboratory was fairly uniform and barometric fluctuations were not excessive, the assimilation and respiration values would not be much affected relatively by converting the carbon dioxide per liter of air to standard conditions of temperature and pressure, or some other standard. Moreover this conversion to some standard would in several respects not be a representative picture of the situation, and assimilation values are given as they were actually obtained.

VARIETIES TESTED.—Experiments were conducted with the leaves of McIntosh, Delicious and seedling apple trees. All the trees were one year old and planted in soil in 5- and 10-gallon galvanized pails. At the time of planting, the nursery stock was cut back to the surface of the soil and only one vigorous shoot allowed to develop from the stub of the scion. The shoots were of varying vigor from 70 to 125 cm. in length and bore leaves ranging from about 45 to 85 cm.^{2} in area. The experiments were carried out before and after the terminal bud had formed on the shoots, but in all cases the leaves under experimentation had attained their full size. The trees grew in the greenhouse under favorable conditions until ready for experimentation when they were brought into the laboratory. During the time they were not being used for experiments, they were placed near the laboratory window with the natural light supplemented by the Mazda lamp on dull days. Soil moisture was kept at an optimum since the water content of the leaf has a marked influence on the rate of assimilation (**8, 18**).

Experimentation

SERIES 1

The purpose of these experiments was to ascertain what effect light intensity which was held constant for each experiment, but varied from experiment to experiment, with a slow and constant rate of air flow, and moderate leaf temperatures, had on the hourly course of assimilation and the total assimilation of the leaf.

One leaf was used in these experiments. The rate of air flow through the leaf chamber was 0.38 liters/cm.^{2}/hr., and the duration of sampling one-half hour. Different light intensities were used, but the light intensity was held

constant throughout each experiment. With the exception of one or two brief periods when the leaf temperature rose to 36° C. leaf temperatures ranged from 25° to 33° C. One experiment was performed each day and the length of an experiment was 8, 9, or 10 hours. In all, 18 experiments were performed and four examples are shown in figure 4 (A, B, C, D).

TABLE I

ASSIMILATION OF LEAF WITH VARIED LIGHT INTENSITY AND UNIFORM AIR FLOW (AIR FLOW 0.38 LITERS/CM.²/HR.)

LIGHT INTENSITY	DURATION OF EXPERIMENT	TOTAL ASSIMILATION	ASSIMILATION ON ONE-HOUR BASIS	AVERAGE ASSIMILATION ON ONE-HOUR BASIS	CO_2 ASSIMILATED PER GM. CAL. LIGHT × 10^4
gm. cal. / cm.²/hr.	*hr.*	*mg. CO_2 / 100 cm.²*	*mg. CO_2 / 100 cm.²/hr.*	*mg. CO_2 / 100 cm.²/hr.*	*mg.*
5.4	10	19.6	1.96	1.75	32.4
	10	16.9	1.69		
	10	14.4	1.44		
	10	19.2	1.92		
10.8	9	19.5	2.17	2.55	23.6
	10	23.0	2.30		
	9	26.6	2.96		
	10	23.8	2.38		
	9	21.9	2.43		
	10	30.8	3.08		
26.4	8	30.5	3.82	3.34	12.7
	9	26.1	2.90		
	10	32.9	3.29		
38.4	10	40.6	4.06	4.03	10.5
	10	33.9	3.39		
	10	46.4	4.64		
97.8	10	35.7	3.57	4.14	4.2
	10	47.0	4.70		

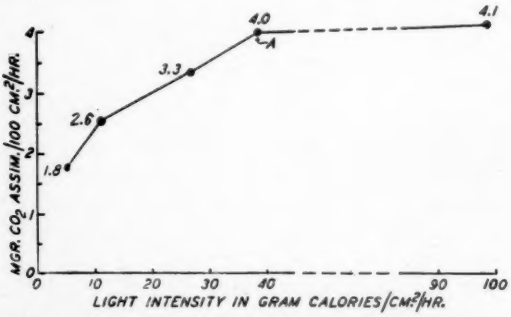

FIG. 3. Effect of light intensity on assimilation with limiting air supply.

In table I are given the experiments performed, their duration and total assimilations. For purposes of comparison, since the experiments are not all of the same length, the average assimilation on an hour basis has been obtained by dividing the total assimilation by the duration of the experiment. The results are shown graphically in figure 3.

SERIES 2

The purpose of these experiments was to ascertain the influence of two rates of air flow on the assimilation of a leaf with constant light intensity and moderate leaf temperatures.

In these experiments one leaf was used. The rates of air flow were 0.38 liters/cm.^{2}/hr. and 1.9 liters/cm.^{2}/hr. Air samples were taken at the beginning of each hour interval. The time of sampling with the higher rate of air flow was 6 min.; with the lower rate, 30 min. Leaf temperatures ranged mostly between 24° and 33° C. One experiment was performed each day and the duration of each experiment was 10 hours. In all, 14 experiments were performed and two examples of the data for the higher rate of air flow are given in figure 4 (E, F).

The total assimilation obtained for each experiment was divided by 10 to obtain the average rate of assimilation per hour and these values for various light intensities and rates of air flow are given in columns 2 and 4 of table II. In columns 3 and 5 are given the averages of the assimilation per hour, and in columns 6 and 7 are given the average milligrams of carbon dioxide assimilated per gram calorie of light received.

TABLE II

ASSIMILATION OF LEAF WITH VARIED LIGHT INTENSITY AND TWO DIFFERENT RATES OF AIR FLOW

LIGHT INTENSITY (GM. CAL./CM.^{2} HR.)	ASSIMILATION ON ONE-HOUR BASIS (MG. CO_2/100 CM.^{2}/HR.)				AVERAGE MG. OF CO_2 ASSIMILATED PER GM. CAL. OF LIGHT			
	AIR-FLOW (0.38 LITERS/CM.^{2}/HR.)		AIR-FLOW (1.9 LITERS/CM.^{2}/HR.)		AIR-FLOW (0.38 LITERS/CM.^{2}/HR.) $\times 10^4$		AIR-FLOW (1.9 LITERS/CM.^{2}/HR.) $\times 10^4$	
gm. cal.	*mg.*	*Average mg.*	*mg.*	*Average mg.*	*mg.*	%	*mg.*	%
12.0	1.29		6.47		11.4	100	48.5	100
	1.44	1.37	5.17	5.82				
20.4	0.74		8.25		7.2	63	44.7	92
	1.80		9.97	9.11				
	2.10							
	1.24	1.47						
37.2	2.46		11.14		6.0	53	35.0	72
	1.98	2.22	14.87	13.01				

Series 3

The purpose of these experiments was to ascertain the influence of light intensity varied hourly during the course of the experiment on the assimilation of the leaf with a constant rate of air flow and moderate leaf temperatures.

One leaf was used in these experiments. The rate of air flow was 1.9 liters/cm.^{2}/hr. and leaf temperatures ranged from 26° to 32° C. Changes in light intensity were made at the beginning of the hour interval and the air samples were taken at the middle of the hour interval. This was done in order to allow the leaf time to adjust its metabolic processes to the changed light intensity. In all experiments, the light intensity was progressively increased to a maximum and then progressively decreased to a minimum. The same sequence of light intensities was used in all experiments. Eight experiments of eight hours duration were performed and the results are given in table III. Two examples are shown in figure 4 (G, H).

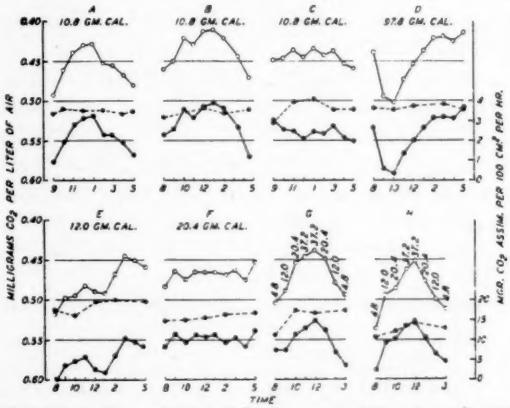

Fig. 4. Effect of various external conditions on assimilation. Solid line with open circles: carbon dioxide content of air passed over leaf (left ordinate); broken line with solid circles: carbon dioxide content of air check (left ordinate); solid line with solid circles: carbon dioxide assimilated per 100 square centimeters of leaf surface per hour (right ordinate). Light intensities given in gram calories per cm.^{2} per hour.

Series 4

The purpose of these experiments was to ascertain the influence of leaf temperature varied hourly during the course of the experiment on assimilation with constant light intensity and rate of air flow.

One leaf was used in these experiments. The leaf temperature was varied by the electric fan, and changes in leaf temperatures were made at the be-

TABLE III

VARIATION IN ASSIMILATION WITH HOURLY VARIATION IN INTENSITY OF ILLUMINATION (AIR FLOW 1.9 LITERS/CM.²/HR.)

EXPERIMENT	LIGHT INTENSITY (GM. CAL./CM.²/HR.)							
	4.8	12.0	20.4	37.2	37.2	20.4	12.0	4.8
	ASSIMILATION (MG. CO_2/100 CM.²/HR.)							
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	5.9	8.4	10.1	11.6	13.5	11.7	7.7	5.1
2	7.5	7.6	11.2	13.0	14.8	11.0	7.2	3.6
3	2.0	9.2	10.3	13.5	14.6	10.3	6.7	4.8
4	0.5	0.2	7.5	15.2	10.3	8.9	12.5	3.8
5	5.5	4.6	6.4	14.0	15.6	15.1	7.6	7.7
6	3.0	7.2	8.5	20.2	16.5	13.4	9.1	8.4
7	3.6	6.8	9.3	13.0	12.0	8.6	4.9	2.7
8	2.8	9.5	7.3	17.9	15.8	12.7	8.1	3.1
Average assimilation	3.9	6.7	8.8	14.8	14.1	11.5	8.0	4.9
Average mg. CO_2 assimilated per gm. cal. of light × 10^4	81.3	55.8	43.1	39.8	37.9	56.4	66.7	102.1
	80%	55%	42%	39%	37%	55%	65%	100%

ginning of the hour intervals. Air samples were taken at the middle of the intervals to allow the leaf to adjust its metabolic processes to the changed leaf temperature. The rate of air flow was 1.9 liters/cm.²/hr. Six experiments, using a temperature range of approximately 30° to 38° C., were performed and two examples are given in figure 5.

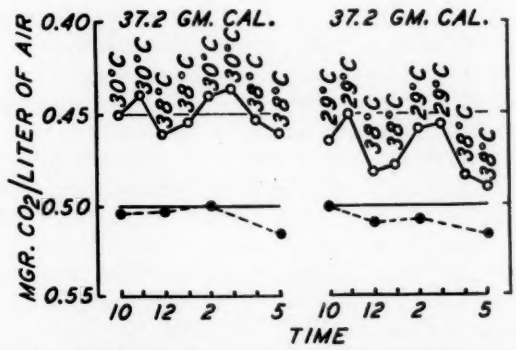

FIG. 5. Effect of leaf temperature on assimilation. Solid line with open circles: carbon dioxide content of air passed over leaf; broken line with solid circles: carbon dioxide content of air checks. Light intensities given in gram calories per cm.² per hour.

Discussion

From series 1 and 2, figure 4 (A to F), it is seen that the assimilation of the apple leaf with uniform light intensity, restricted leaf temperature variation, adequate water supply, and a slow constant rate of air flow was very irregular from hour to hour on some days and fairly constant on others. In general the assimilation starts out relatively low and increases with one, two, or several maxima during the experiment. In some experiments the assimilation subsides and reaches a relatively low value again toward the close of the experiment.

It would be difficult to designate specific external factors that might operate to cause such wide fluctuations in assimilation under these fairly uniform external conditions. Although the lamp used to illuminate the leaf was supplied with 60 cycles A.C. per second, it seems unlikely that the resultant fluctuating illumination would cause any more appreciable difference in the response of the leaf than if it were under steady illumination. McAlister (19) has shown that with high frequencies such as 1/60-second periods of light and darkness, the induction period is very small, and the assimilation practically the same as under steady illumination.

While fluctuations in the carbon dioxide content of the air would be expected to influence assimilation, the absence of correlation between the fluctuations in assimilation and carbon dioxide content of the atmosphere indicates that other factors predominate. This is also indicated by the results of series 3, table III, where assimilations obtained with the same light intensity and external conditions show considerable variation within an experiment and from day to day. Heinicke and Hoffman (10) found that with individual apple leaves there were violent fluctuations in assimilation from day to day which could not always be correlated with prevailing external conditions. They state: "On the whole, the internal conditions which govern the supply of water and nutrients and the translocation and utilization of assimilated materials seem to have a profound influence on the efficiency of the foliage of the apple as well as that of other plants." [See also Heinicke (7), Heinicke and Childers (8), Childers and Cowart (3).] Heinicke and Childers (9), working with the foliage of an entire tree, conclude that while variations in carbon dioxide concentration may have some influence in determining the rate of apparent photosynthesis, other factors are more important under natural conditions. Kostytschew and his coworkers (14, 15, 16, 17) have pointed out that the directly limiting factors are probably internal and such factors are subject to varying degrees of stimulation by light and temperature, and so on, depending on the time of the day or the condition of the tissue. [See also Boonstra (2), Tseschnokov and Bazyrina (21).] Harder (5) and Arnold (1) have shown that in some plants assimilation will fall off as a result of fatigue even though light, temperature, and carbon dioxide of the air remain constant.

From series 2, table I and figure 3, there is a very apparent increase of the average rate of assimilation per hour with increase in light intensity up to 38.4 gm.cal./cm.^{2}/hr. (fig. 3, A), but for a light intensity of 97.8 gm.cal./cm.^{2}/hr., there is very little further increase. It seems likely that the rate of air flow, 0.38 liter/cm.^{2}/hr., becomes limiting for this high light intensity. HEINICKE and HOFFMAN (**10**) have shown that the maximum rate of assimilation of apple leaves on clear days (where the light intensities were probably not greater than 97.8 gm.cal./cm.^{2}/hr.) is considerably limited by rates of air supply less than 1 liter/cm.^{2}/hr., and CHRISTOPHER (**4**) also working with apple leaves has found that an air supply has a marked limiting influence on assimilation especially where low air supplies in the neighborhood of 1 liter/cm.^{2}/hr., or less, are used. In table I it is seen that the average milligrams of carbon dioxide assimilated per gm. cal. of light decrease as the light intensity increases. From the rapidity with which these values decrease, it seems probable that the air flow of 0.38 liter/cm.^{2}/hr. becomes appreciably limiting even at the lower light intensities.

The results of series 2, figure 4 (E, F), show irregular fluctuations in assimilation as in series 1, figure 4, (A to D). Calculation of the ratios between average hourly assimilations at corresponding light intensities in table II gives the values 4.3, 6.2, and 5.9. Roughly the ratio is in the region of 5, the ratio of the two rates of air flow. The results illustrate the importance of an adequate air supply to the leaf and indicate that the air flow of 0.38 liter/cm.^{2}/hr. was limiting. It is likely that the air flow of 1.9 liters/cm.^{2}/hr. was also limiting. This conclusion is supported by the results of HEINICKE and HOFFMAN (**10**) and CHRISTOPHER (**4**), which indicate that an air supply of at least 2.5 liters/cm.^{2}/hr. is necessary to simulate natural conditions in photosynthetic studies. From table II, columns 6 and 8, it is seen that the mg. of carbon dioxide assimilated per gm. cal. of light decrease for both rates of air flow as the light intensity is increased. If, however, 100 per cent. is arbitrarily assigned to the values obtained for the light intensity of 12.0 gm.cal./100 cm.^{2}/hr. and the other values expressed as percentages of these values, it is seen that the percentages decrease more rapidly for the lower rate of air flow. Evidently the limiting action of the lower rate of air flow becomes increasingly greater over the higher rate of air flow as the light intensity is increased.

From the above results it is apparent that the ranges of air supply and light intensity used in this study are limiting factors. HOOVER, JOHNSTON, and BRACKETT (**11**), working with wheat under carefully controlled external conditions, observed that there is a limited transition region over which both the carbon dioxide concentration and light intensity seem to be limiting factors. While they kept their rate of air flow to the plant constant, and varied the carbon dioxide concentration of the air instead of the reverse as was done

in the above experiments, both methods are means of carbon dioxide supply and the results seem to be comparable. They ascribe this transition region to a lack of homogeneity of light intensity and carbon dioxide concentration throughout the leaf which may explain, in part, the results above.

The response of assimilation to changes in light intensity is shown in series 3, figure 4 (G, H), and table III. The averages of the assimilations obtained for each light intensity, taken in the order in which they were used in the experiments, indicate that in general there is a response in assimilation to changes in light intensity, and that this adjustment of assimilation to light intensity takes place within an hour interval.

There is also fair agreement in average assimilations obtained for corresponding light intensities and rates of air flow in tables II and III. A close relationship between photosynthesis and illumination in hour intervals has been observed by THOMAS and HILL (**20**) for wheat and alfalfa. Responses in the assimilation of apple leaves to changes in light intensity over longer periods of time have been observed by HEINICKE and HOFFMAN (**10**) for individual leaves and HEINICKE and CHILDERS (**9**) for tree foliage. MCALISTER (**19**), working with wheat under carefully controlled external conditions, has obtained short induction periods of a few minutes for illumination, furnishing additional evidence of the rapidity of response of the assimilatory mechanism to changes in light intensity.

Series 4, figure 5, indicates that high leaf temperatures such as 37° or 38° C. result in a reduced assimilation of the leaf as compared with temperatures around 30° C. For individual apple leaves HEINICKE and HOFFMAN (**10**) find that higher temperatures may, under certain conditions, reduce the rate of apparent photosynthesis. They find that the critical temperature of the apple leaf is about 35° C. which appears to be in accord with the above data. For apple foliage HEINICKE and CHILDERS (**9**) find that high temperatures cause a reduction in assimilation. They conclude that the rate of respiration is influenced more by temperature than is the rate of photosynthesis, the higher temperatures favoring higher respiration with a reduction in the rate of apparent photosynthesis. They also advance the possibility of higher temperatures interfering with the photosynthetic mechanism and directly reducing assimilation. THOMAS and HILL (**20**) note that at times the photosynthetic maximum is appreciably higher than normal when the sun is intermittently obscured by clouds for a few seconds to one or two minutes. They attribute it in part to a lower leaf temperature with attendant lower respiration and possibly in part to the orientation of the chloroplasts. MCALISTER (**19**) obtained progressive increases in respiration with temperatures of 12°, 21°, and 31° C., and found that respiration was independent of light intensity. From the apparatus and technique employed it seems very likely that the respiration values he obtained represent the respiration actually tak-

ing place during photosynthesis. It seems likely in the experiments reported here that the reduction in apparent assimilation at the higher temperatures was in part, at least, caused by increased respiration with possible inhibition of the photosynthetic mechanism.

Summary

1. Apple leaves were subjected to various external conditions in the laboratory and determinations of the rate of assimilation made in hour periods.

2. The results indicate that under fairly uniform external conditions, the rate of assimilation of apple leaves is irregular. On some days the rate of assimilation is fairly uniform and on other days it fluctuates appreciably from hour to hour and may have several maxima in the course of a day. It seems probable that internal factors play a significant and important part in the assimilation of the apple leaf.

3. The results indicate that under fairly uniform external conditions the response in assimilation of the apple leaf to changes in light intensity take place within an hour.

4. Rates of air flow of 0.38 and 1.9 liters per cm.^{2} of leaf surface per hour used with low and moderate light intensities gave rates of assimilation for the same light intensities that were roughly in proportion to the ratio of the rates of air flow. The limiting action of the lower rate of air flow became increasingly greater over the higher rate of air flow as the light intensity was increased. Further studies indicated that the lower rate of air flow became almost completely limiting at high light intensity.

5. The results obtained with the rates of air flow and light intensities used in these studies indicate that in the assimilation of the apple leaf there is a transition range over which light intensity and rate of air flow are both limiting factors.

6. In general, under fairly uniform external conditions, leaf temperatures of about 38° C. seemed to result in reduced assimilation as compared to leaf temperatures of about 30° C. It is probable that this reduction in assimilation at higher leaf temperatures was due in part, at least, to increased respiration with possible inhibition of the photosynthetic mechanism. The results seem to support the conclusion of other investigators that the critical temperature of the apple leaf is about 35° C.

The author is indebted to Professor A. J. HEINICKE of the Department of Pomology, Cornell University, in whose laboratory and under whose general direction this work was carried out.

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INFLUENCE OF CERTAIN AMINO ACIDS AND OF NICOTINIC ACID UPON THE NICOTINE CONTENT OF TOBACCO LEAVES[1]

RAY F. DAWSON

Introduction

The tobacco alkaloid nicotine possesses a molecular structure by which it may be related empirically to the amino acid proline. Proline is α-pyrrolidinecarboxylic acid. Nicotine may be considered as an N-methylated proline containing a β-pyridine ring attached to the alpha position in place of the carboxyl. These similarities have been pointed out by TOTTINGHAM (**9**) and have been employed by TRIER (**12**) as a basis for extensive speculations on the probable origin of nicotine in the tobacco plant. According to TRIER, nicotine may be formed *in vivo* from proline and nicotinic acid by a simultaneous oxidative decarboxylation of the two molecules. The methylation of the pyrrolidine nitrogen was accounted for by the action of methyl alcohol as a dismutation product of formaldehyde. TRIER postulated the formation of the pyridine portion of the nicotine molecule from proline through the intervention of formic acid as the second dismutation product of formaldehyde. In short, the hypothesis of TRIER assigned to proline the rôle of precursor of both the five- and the six-membered rings of the nicotine molecule. It should be noted, however, that TRIER'S theory was advanced at a time when nicotinic acid was practically unknown in the literature of plant chemistry.

KLEIN and LINSER (**7**) tested TRIER'S hypothesis by culturing the excised leafy shoots of tobacco plants in dilute aqueous solutions of proline. These investigators obtained large increases in the nicotine content of the leaves and stems of the plants to which proline had been supplied. The hydrochlorides of glutamic acid and of ornithine were found to possess similar effects. GORTER (**5**) critically reviewed the work of KLEIN and LINSER and presented data of his own to show that both those plants provided with proline and those cultured in KNOP'S solution after a period of 4 or 5 days contained less nicotine than at the beginning of the experiment. The increase in nicotine content of the leaves of the plants supplied with proline was only apparent and represented merely a less extensive decrease rather than an actual synthesis of nicotine. In the present study attention was given to the influences of proline, pyrrolidonecarboxylic acid, glutamic acid, ornithine, nicotinic acid, and other substances upon the nicotine content of tobacco leaves by the use of methods of culture and of expression of data

[1] Contribution from the Osborn Botanical Laboratory, Yale University.

differing in certain respects from those employed by KLEIN and LINSER and by GORTER.

Materials and methods

Tobacco seedlings of the Havana Seed and Connecticut Broadleaf no. 38 varieties were grown in white quartz sand to which a modified SHIVE three-salt nutrient solution was added at daily intervals. The composition of this solution was calcium nitrate 0.0042, monopotassium phosphate 0.0021, and magnesium sulphate 0.0042 partial gram moles per liter. Supplements of iron, boron, manganese, and copper were added in the proportions of two parts per million. The seedlings were transplanted when about one inch in height to a well composted soil in eight-inch porous clay pots. Weekly applications of a 0.1 per cent. suspension of Nitrophoska (15–30–15), extension of the daily photoperiod by a series of 150-watt Mazda lamps, and an average greenhouse temperature of 25° C. made possible the production of fairly vigorous plants with large dark green leaves even during the winter season. In all cases the plants were grown in the greenhouse between the months of September and June.

When the plants possessed 10 to 15 large leaves each, they were carefully selected for uniformity in height of stem and leaf development. In general, from 4 to 6 plants were used in each experimental unit. An initial leaf sample and a final leaf sample taken from normal rooted plants at the beginning and the end of each experiment provided supplementary controls and served to indicate the course of nicotine synthesis under normal conditions of plant growth.

At the end of the experimental period the leaves were taken from the stems and placed in a refrigerator to await preparation for drying. The midribs were removed and the leaf blades immediately dried in a forced current of air preheated to 80° C. Drying was usually complete within an hour, but the tissues were allowed to remain in the oven for 2 hours. The material was then pulverized, weighed, and preserved in tightly stoppered bottles. The results of subsequent chemical analyses are expressed on the basis of this roughly dried material.

The cut stem method of plant culture as employed by KLEIN and LINSER (7) in their studies on nicotine metabolism was adapted for large scale use in the present investigation. The details of this modified procedure have been described elsewhere (3). Essentially, the technique involves the use of low temperatures to prevent decomposition of the culture solutions by microorganisms. The necessity of sterilizing plant tissues by chemical means and of autoclaving heat unstable organic compounds was thus avoided. The culture solutions were made up by dissolving the desired quantities of material in tap water and adjusting the acidity to about pH 3.0.

Nicotine was determined by steam distillation from alkaline solution (**1**). Duplicate or triplicate determinations were made for each sample of tissue. Approximately 2 grams of dried leaf were used for each distillation, and 750 ml. of distillate were collected from each sample.

All data from these experiments are expressed in terms of milligrams of nicotine in the leaves of the individual plant. All changes in volatile alkaloid content amounting to less than 20 per cent. are arbitrarily considered to be within the limits of experimental error and hence of no value in establishing the premises of this study.

Glutamic acid was prepared by the addition of one equivalent of potassium hydroxide to a solution of *d*-glutamic acid hydrochloride. The precipitate was filtered, washed with water, and dried. Pyrrolidonecarboxylic acid was obtained by autoclaving a 10-per cent. suspension of glutamic acid prepared as previously described at 115 pounds pressure for 6 hours (**11**). Since no ready method was available for the determination of the components of the resulting equilibrium mixture, it was assumed for purposes of making up the culture solutions that the reaction had proceeded to completion. All the amino acids, sugars, and other compounds and reagents used in this study beyond those whose preparations have already been described were the highest grades obtainable from Merck and Co., Inc., and from the Eastman Kodak Co.

Nicotine was isolated as the dipicrate from the steam distillate of each sample showing a significant deviation from the controls with respect to nicotine content. The purpose of this operation was to determine from the melting point of each preparation whether or not the increase in nicotine content in the tissues as determined by the steam distillation method represented an actual synthesis of nicotine itself or merely a conversion of the substances supplied in the culture solutions into steam volatile bases which could be precipitated with silicotungstic acid and, hence, be determined as nicotine. The possibility of such interference seemed especially likely in the case of proline and nicotinic acid, both of which could conceivably be decarboxylated within the leaf tissues to yield pyrrolidine and pyridine respectively. The melting point of once-recrystallized nicotine dipicrate isolated from a sample of normal leaf tissue was 221–222° C. (uncorrected).

Results

The effects of glutamic acid and of pyrrolidonecarboxylic acid upon the nicotine content of tobacco leaves

Trier (**12**) related glutamic acid and its lactam pyrrolidonecarboxylic acid to nicotine synthesis through proline. Theoretically, it is possible that glutamic acid may give rise to proline in plant tissues by the reactions glutamic acid → pyrrolidonecarboxylic acid → proline. The first step is easily

carried out in the laboratory by heating an aqueous solution of glutamic acid as has been described. The reduction of the carbonyl oxygen of pyrrolidonecarboxylic acid to the CH_2 of pyrrolidinecarboxylic acid (proline) is much more difficult to accomplish (**4**). In the present study two experiments were conducted employing both glutamic acid and pyrrolidonecarboxylic acid in the culture solutions.

In the first experiment, 24 Connecticut Broadleaf no. 38 tobacco plants were placed, in the manner already described for cut stem cultures, in 0.007 M solutions of *d*-glutamic acid, *l*-pyrrolidonecarboxylic acid, and citric acid all adjusted to pH 3.4. The flasks containing the leafy shoots were placed in a diffusely lighted room the relative humidity of which remained about 35 per cent. The solutions were maintained at a temperature of about 10° C. After 96 hours of culture the plants were harvested and dried. The quantities of solution absorbed, the dry weights, and the data for nicotine content are found in table I.

TABLE I

EFFECTS OF *d*-GLUTAMIC ACID, *l*-PYRROLIDONECARBOXYLIC ACID, AND OF CITRIC ACID ON THE NICOTINE CONTENT OF TOBACCO LEAVES AFTER CUT STEM CULTURE FOR 96 HOURS. LEAVES HARVESTED MARCH 27, 1937

EXPERIMENTAL GROUP	DRY WEIGHT OF LEAVES PER PLANT	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT	INCREASE IN NICOTINE	VOLUME OF SOLUTION ABSORBED PER PLANT
	gm.	%	*mg.*	%	*ml.*
Initial controls	4.9	1.62	79	+10	
Water	4.9	1.47	72	0	270
Pyrrolidonecarboxylic acid	5.0	1.80*	90	+25	313
Glutamic acid	5.3	1.40	74	+ 3	465
Citric acid	4.8	1.43	69	− 4	321
Final controls (rooted plants)	5.4	1.50	81	+13	

* Once-recrystallized picrates from the steam distillate melted at 221–222° C. (uncorrected).

The results indicate an increase in absolute nicotine content only in the leaves of those plants which had been supplied with pyrrolidonecarboxylic acid. This increase amounted to nearly 25 per cent. of the quantity of volatile alkaloid present in the leaves of the control plants in water. Glutamic acid exerted no influence on nicotine formation, but there were unmistakable indications of its effects on solution absorption and leaf turgidity (**3**). Citric acid also failed to alter the process of nicotine synthesis.

A second experiment employing *d*-glutamic acid and *l*-pyrrolidonecarboxylic acid was conducted under the same conditions as were described for the preceding experiment. The plants used were similar with respect to

size and stage of development, and the culture solutions were the same except that glycine was substituted for citric acid and that all the solutions were adjusted to pH 3.0. Each experimental unit consisted of 5 Connecticut Broadleaf no. 38 tobacco plants. After 100 hours of culture the plants were harvested and the leaves dried. The dry weights and analytical data are presented in table II A.

TABLE II

EFFECTS OF *d*-GLUTAMIC ACID, *l*-PYRROLIDONECARBOXYLIC ACID, AND OF GLYCINE UPON THE NICOTINE CONTENT OF TOBACCO LEAVES AFTER CUT STEM CULTURE

EXPERIMENTAL GROUP	DRY WEIGHT OF LEAVES PER PLANT	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT	INCREASE IN NICOTINE	VOLUME OF SOLUTION ABSORBED PER PLANT
	gm.	%	*mg.*	%	*ml.*
(A) Culture period 96 hours. Leaves harvested April, 6, 1937					
Initial controls	6.1	1.50	92	+ 2	
Water	6.1	1.47	90	0	330
Pyrrolidonecarboxylic acid	6.6	1.84	121*	+35	346
Glutamic acid	6.4	1.54	99	+10	432
Glycine	6.0	1.72	103	+14	362
Final controls (rooted plants)	6.3	1.57	99	+10	
(B) Culture period 40 hours. Leaves harvested April 17, 1937					
Water	6.46	1.11	72	0	210
Glutamic acid	6.39	1.45	93	+29	396

* Once-recrystallized picrates from the steam distillate melted at 221–222° C. (uncorrected).

Again pyrrolidonecarboxylic acid gave a significant increase in absolute nicotine content: this time the increase was 35 per cent. above the corresponding value for the leaves of the plants in water. Glutamic acid, however, failed to influence nicotine formation in spite of the marked physiological influence which it again exhibited upon the plants to which it was supplied. Glycine gave an apparent increase of 14 per cent. in nicotine. The order of magnitude of this variation, however, lies well within the limits of experimental error and cannot be regarded as significant.

The evidence of physiological activity exhibited by glutamic acid in the two experiments just described led to the suggestion that the quantity of the acid supplied to the plants may have been inadequate for nicotine formation from the acid as a precursor because of its rapid utilization in other cell reactions of considerably greater intensity. Consequently, an experiment was conducted in which twice the concentration of glutamic acid was em-

ployed in the culture solutions. Ten plants (Connecticut no. 38) were placed with their cut stems in 0.014 M solutions of *d*-glutamic acid and in water, both adjusted to pH 3.2. Room temperature remained around 22° C., and the light was diffuse. At the end of 40 hours all the plants were harvested and the leaves dried.

Apparently in close agreement with the suggestions advanced above, the analytical results (table II B) indicate a synthesis of alkaloid in those leaves supplied with glutamic acid to the extent of 29 per cent. above the amount present in the leaves of the plants in water. Such an increase, although apparently well above the limits of arbitrarily established experimental error, is considered insufficient alone to establish a relationship between this amino acid and the process of nicotine synthesis. Indeed, glutamic acid is sufficiently far-removed from the end product of TRIER's scheme that no very decisive or consistent results might be expected from its use in experiments of this type.

The results for the influence of pyrrolidonecarboxylic acid upon nicotine synthesis, however, seem to be much more suggestive. From the evidence at hand and that of KLEIN and LINSER (**6**) upon the synthesis of trigonelline from pyrrolidonecarboxylic acid, it appears that some mechanism may be available in the leaf cells by which this substance can be converted into nicotine or related compounds.

THE EFFECT OF PROLINE UPON THE NICOTINE CONTENT OF TOBACCO LEAVES

The third step in the hypothetical nicotine synthesis of TRIER (**12**) involves the simultaneous oxidative decarboxylation of a molecule of n-methyl proline and a molecule of nicotinic acid with subsequent combination of the cyclic residues to give the characteristic structure of the nicotine molecule. It should be noted that the substance which would be formed in this reaction from proline itself with nicotinic acid is identical with one of the minor tobacco alkaloids closely associated with nicotine, namely nornicotine (**8**). KLEIN and LINSER (**7**) reported the results of eight different experiments in which proline alone gave an average increase of about 80 per cent. in the nicotine content of the green tobacco plants. Their conclusion agrees in principle with the hypothesis of TRIER that proline acts as the precursor not only of the pyrrolidine but also of the pyridine ring structures of the nicotine molecule.

In the first attempt to repeat the work of these authors, 24 Connecticut no. 38 tobacco plants were placed under conditions of cut stem culture in 0.1 per cent. solutions of *d*-arginine monohydrochloride, *l*-proline, *d l*-α-amino-*n*-valeric acid, nicotinic acid hydrochloride, and sodium magnesium

TABLE III

EFFECTS OF *l*-PROLINE AND OF OTHER SUBSTANCES UPON THE NICOTINE CONTENT OF TOBACCO LEAVES AFTER CUT STEM CULTURE FOR 96 HOURS.
LEAVES HARVESTED JUNE 14, 1937

EXPERIMENTAL GROUP	DRY WEIGHT OF LEAVES PER PLANT	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT	INCREASE IN NICOTINE	VOLUME OF SOLUTION ABSORBED PER PLANT
	gm.	%	*mg.*	%	*ml.*
Initial controls	9.2	1.15	106	+13	
Water	7.1	1.33	94	0	190
Sodium magnesium chlorophyllin		toxic			
α-Amino-*n*-valeric acid	7.4	1.33	98	+ 4	137
Arginine monohydrochloride	7.1	1.38	98	+ 4	150
Nicotinic acid hydrochloride	8.0	1.54	123*	+31	500
Proline	7.4	1.56	115†	+22	200
Final controls (rooted plants)	11.5	1.06	121	+29	

* Once-recrystallized picrates from the steam distillate melted at 221–222° C. (uncorrected).

† Once-recrystallized picrates from the steam distillate melted at 220–221° C. (uncorrected).

chlorophyllin[2] and in water. The solutions were chilled to 16° C. by use of running water. The light was diffuse and indirect, and the room temperature remained between 22° and 23° C. The leaves were harvested and dried at the end of the fourth day, at which time only the plants supplied with nicotinic acid hydrochloride were still fresh and turgid. The plants which had been placed in solutions of sodium magnesium chlorophyllin wilted within 2 hours after the inception of the experiment and were hence discarded.

Two of the substances employed in the culture solutions in this experiment induced significant variations in the nicotine content of the leaves. *l*-Proline apparently increased the absolute nicotine content of the leaves 22 per cent. above the quantity of the alkaloid present in those leaves cultured in water. Nicotinic acid hydrochloride exerted a similar influence (table III). The magnitude of this figure for the effects of proline on nicotine content lies very close to the limits of experimental error. A second experiment was conducted, therefore, to serve as a check on the preceding one.

Twenty Connecticut no. 38 plants were placed with their stems in 0.014 M solutions of glucose, glucose plus ammonium chloride, and glycine, and in

[2] Sodium magnesium chlorophyllin was supplied through the courtesy and kindness of DR. F. M. SCHERTZ of the American Chlorophyll Co., Alexandria, Va.

0.1 per cent. solutions of *l*-proline and *d*-arginine monohydrochloride at pH 3.0. These plants were in the period of most rapid growth during the month of May, and, hence, probably represented the nearest approach to carbohydrate-high summer plants of any used in this study. Throughout the experiment the room temperature fluctuated between 20° and 25° C., while the relative humidity remained around 67 per cent. Light was indirect and diffuse. After 70 hours the leaves were harvested and dried.

An unmistakable increase of 50 per cent. in the total nicotine content of the leaves followed the absorption of proline. Glycine, glucose, glucose plus ammonium chloride, and arginine, respectively, failed to exert a significant influence on nicotine synthesis or utilization under the conditions of this experiment. The order of magnitude of the increase induced by proline in this instance seems to lie well outside the limits of experimental error and likewise seems to provide a very good check against the results obtained in the preceding experiment. The data for this experiment are found in table IV.

TABLE IV

EFFECTS OF *l*-PROLINE AND OF OTHER SUBSTANCES UPON THE NICOTINE CONTENT OF TOBACCO LEAVES AFTER CUT STEM CULTURE FOR 69 HOURS. LEAVES HARVESTED JUNE 13, 1937

EXPERIMENTAL GROUP	DRY WEIGHT OF LEAVES PER PLANT	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT	INCREASE IN NICOTINE	VOLUME OF SOLUTION ABSORBED PER PLANT
	gm.	%	*mg.*	%	*ml.*
Initial controls	5.16	1.00	62	+ 4	
Glucose	4.50	1.11	50	0	430
Glucose plus ammonium chloride	4.56	1.16	53	+ 6	430
Glycine	4.26	1.06	45	−10	485
d-Arginine monohydrochloride	4.50	1.08	49	− 2	267
l-Proline	4.98	1.50	75*	+50	233
Final controls (rooted plants)	5.88	1.11	65	+30	

* Once-recrystallized picrates from the steam distillate melted at 221–222° C. (uncorrected).

Taken as a whole, the data from this study and from that of KLEIN and LINSER seem to establish the fact that tobacco leaf tissues have the capacity for transforming the pyrrolidine nucleus of proline into nicotine. Inspection of the melting point values for the picrates isolated from the distillation mixtures indicates that they were identical with the melting point of nearly pure nicotine dipicrate. Hence, although it is barely possible that picrates of such other substances as pyrrolidine or pyridine might have escaped detection by means of greater solubility in the aqueous recrystal-

lizing medium, it seems reasonable to assume that the differences in volatile alkaloid content of the leaf samples employed in this study might be safely attributed to increased synthesis of nicotine itself.

One other important aspect of the data should be considered. The work of GORTER (5) appears to show that increases in nicotine content obtained by the use of proline were caused by a less extensive decrease in nicotine content rather than by an actual increase of alkaloid in the plant tissues. This investigator reported losses during water culture in the nicotine content per unit leaf area of an order of magnitude considerably in excess of the values obtained in the extensive investigations of VICKERY *et al.* (10). In the present study small losses of volatile alkaloid have been almost invariably encountered after periods of cut stem culture, but in no case have losses exceeding 10 to 15 per cent. been observed. Since it seems reasonable to assume that a similar loss occurred in the leaves of all the experimental groups regardless of the solutes supplied, the values for percentage increase or decrease in nicotine content have been calculated on the basis of the number of milligrams of nicotine in the leaves of the plants supplied with tap water. In all cases in which significant increases in nicotine content have been observed in this study, the total quantity of the alkaloid in the leaves has exceeded that present in similar samples of leaves collected and analyzed at the inception of the experiments, and in most cases has exceeded the amount present in the leaf samples collected at the ends of the experimental periods. Therefore, the results of the present investigation seem to confirm the findings of KLEIN and LINSER with respect to the ability of proline to increase the nicotine content of the leaves of excised tobacco plants.

THE INFLUENCE OF NICOTINIC ACID UPON THE NICOTINE CONTENT OF TOBACCO LEAVES

Assuming that excised tobacco leaves are able to synthesize nicotine directly from proline, it would seem that the supply of proline should be a limiting factor in the synthesis of the alkaloid. Furthermore, it would appear that the pyridyl portion of the nicotine molecule must necessarily be provided by the conversion of a portion of the proline itself into nicotinic acid, as suggested by TRIER and by KLEIN and LINSER, or by preformed nicotinic acid as a naturally occurring constituent of tobacco leaf tissues. In the event TRIER'S hypothesis has a basis in fact, therefore, the substitution of nicotinic acid for proline in the culture medium would be expected to have no influence upon the nicotine content of the leaves, unless, of course, the assumed reaction proline → nicotinic acid were reversible. Since KLEIN and LINSER do not report the results of investigations on this phase of the problem, experiments were designed to check these assumptions.

Four Connecticut no. 38 tobacco plants were placed with their cut stems

in 0.1 per cent. solutions of nicotinic acid hydrochloride. Four similar plants were placed in tap water and the entire group of cultures placed in sinks containing running water at 16° C. On the third day after the inception of the experiment, the lower leaves of the plants in water had begun to wilt. The plants in solutions of nicotinic acid hydrochloride, however, were extremely turgid and were absorbing solution at a rapid rate. At 100 hours the experiment was ended because of the complete wilting of the plants in water culture. No change was detected in the condition of the plants in nicotinic acid solutions. The leaves of the latter plants still retained the remarkable turgidity and freshness that had characterized them from the beginning of the experiment (**2**). In this respect, the effects of nicotinic acid upon physiological processes of tobacco leaves seemed even more pronounced than the somewhat similar effects of glutamic acid which were described earlier.

A comparison of the relative nicotine contents (table III) of the leaves receiving nicotinic acid shows an increase of nearly 16 per cent. at the end of the experimental period presumably due to the presence of the acid. The absolute quantity of volatile alkaloid present in the latter case, however, was 31 per cent. above that present in the leaves in water culture: the difference was apparently accounted for by a materially lessened dry weight loss during the culture period. It should be observed that the amount of alkaloid present in the leaves of these plants at the end of the experiment exceeded the amount present in the leaves of normal rooted plants at the end of the same period.

Further data on this phase of the problem were given by the following series of cut stem cultures. These experiments were conducted in direct light in the greenhouse in December. The previous experiments had been conducted in diffusely lighted laboratory rooms. Twelve Havana Seed tobacco plants were cut from their roots and placed in jars containing 0.013 M nicotinic acid hydrochloride plus 0.008 M proline. Two sets of 12 plants each were placed in 0.013 M solutions of nicotinic acid hydrochloride without the addition of proline. The solutions were kept chilled to a temperature of about 3° C. by continuous applications of cracked ice. The experiment was ended after the lapse of 48 hours.

Increases of 26, 26, and 32 per cent., respectively, were observed in the absolute quantity of nicotine present in the leaves after culture in solutions containing nicotinic acid hydrochloride (table V). The addition of proline to one of these culture solutions seemed in no way to influence the trend of nicotine synthesis. Dry weight increased in all of the plants under the influence of direct light exposure. The interesting feature of these results lies in the fact that the relative content of nicotine in terms of percentage of dry weight remained about constant: the increase in absolute nicotine

TABLE V

EFFECT OF NICOTINIC ACID HYDROCHLORIDE UPON THE NICOTINE CONTENT OF TOBACCO LEAVES AFTER CUT STEM CULTURE FOR 48 HOURS.
LEAVES HARVESTED DECEMBER 9, 1937

EXPERIMENTAL GROUP	DRY WEIGHT OF LEAVES PER PLANT	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT	INCREASE IN NICOTINE	VOLUME OF SOLUTION ABSORBED PER PLANT
	gm.	%	*mg.*	%	*ml.*
Initial controls	2.8	1.62	45	− 4	
Water (no. 1)	3.1	1.51	47	0	93
Water (no. 2)	2.9	1.42	41	−13	103
Nicotinic acid HCl plus proline	4.1	1.45	59	+26	161
Nicotinic acid HCl (no. 1)	4.3	1.45	62	+32	140
Nicotinic acid HCl (no. 2)	4.1	1.45	59	+26	147
Final controls (rooted plants)	3.5	1.60	56	+19	

content in every case was directly associated with growth and increase in dry weight. Since the present experiment was conducted for only half the time usually allotted this type of experiment, it is impossible to know whether or not the action of nicotinic acid would have eventually led to an increase in the relative amount of nicotine throughout the tissues of the entire plant. It is, of course, obvious that such a condition must prevail before it can be assumed that nicotinic acid itself is capable of entering into a direct synthesis of nicotine.

From the evidence at hand, it appears that nicotinic acid possesses the ability to increase the quantity of nicotine in the leaves of excised tobacco plants. Preliminary observations, however, indicate that this relationship is not of the same type as that represented by proline and by pyrrolidonecarboxylic acid and possibly by glutamic acid. Nicotine synthesis in the presence of nicotinic acid seems to be closely related to growth and dry weight accumulation. Therefore, the increases in nicotine content may not represent a passive synthesis from nicotinic acid as a precursor but an indirect synthesis in which nicotinic acid enters into one or more important metabolic reactions before it is converted into nicotine. The similarity of this induced nicotine synthesis to the normal process of alkaloid formation in the tobacco leaf suggests important possibilities for approaching the problem of the physiological significance of nicotine in the nitrogen metabolism of tobacco.

Summary

1. The influence of various naturally occurring organic compounds on the nicotine content of green tobacco leaves was studied by culturing excised

tobacco shoots in aqueous solutions of these substances. This was followed by quantitative analyses of the leaf tissues for changes in nicotine content.

2. The substances tested for their influences on nicotine formation were *l*-proline, *d*-glutamic acid, *l*-pyrrolidonecarboxylic acid, sodium magnesium chlorophyllin, glycine, *d l*-α amino-*n*-valeric acid, *d*-arginine monohydrochloride, nicotinic acid hydrochloride, *d*-glucose, and citric acid.

3. Proline, nicotinic acid hydrochloride, pyrrolidonecarboxylic acid, and glutamic acid gave indications in varying degree of possessing an effect upon the nicotine content of tobacco leaves. These results appear to confirm the observations of KLEIN and LINSER (7) and partially to support the hypothesis of TRIER (**12**).

4. In so far as they may be compared, the effectiveness of each of these substances in increasing nicotine content paralleled the respective closeness of its molecular structure to the nicotine molecule. That is, glutamic acid was least effective and is most remotely related to nicotine in chemical structure. Pyrrolidonecarboxylic acid was moderately effective and is somewhat more closely related to a portion of the nicotine molecule in chemical structure. Proline was most effective and is closely related structurally to one portion of the nicotine molecule. Nicotinic acid was also effective and is somewhat closely related to another portion of the nicotine molecule in chemical structure, but its effectiveness was, in these experiments at least, due almost entirely to its influence on growth and dry weight accumulation of tobacco leaves during cut-stem culture. In part, the results of this study again appear to substantiate the theory of TRIER with regard to the mechanism of nicotine synthesis *in vivo*.

5. It was shown by isolation and melting point determinations of the picrates of the volatile alkaloid complex of the various samples showing a significant increase in nicotine content that the analytical differences were probably due to increases in nicotine itself rather than to decomposition products of the substances supplied to the leaves.

6. Sodium magnesium chlorophyllin, glycine, *d*-arginine monohydrochloride, *d*-glucose, citric acid, and *d l*-α-amino-*n*-valeric acid gave no indications of possessing an influence upon the nicotine content of tobacco leaves.

7. Two of these substances markedly influenced the dry weight accumulation, turgidity, and growth of tobacco leaves during cut stem culture. *d*-Glutamic acid appeared to possess a sparing action on dry weight loss of tobacco leaves during cut stem culture in diffuse light and to greatly increase the water uptake and turgidity of these plants. This effect was independent of the influences of glutamic acid on nicotine content. Nicotinic acid possessed an even more marked stimulus for growth, dry weight accumulation, and increased turgidity, but this influence was associated in every case with an increase in the nicotine content of the leaves.

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GROWTH OF WHEAT PLANTS FROM DRY AND SOAKED IRRADIATED GRAINS

EDNA LOUISE JOHNSON

(WITH FIVE FIGURES)

This comparative study of plant growth from dry and soaked irradiated seeds is in agreement with earlier experiments of the writer and of others (1) with regard to the greater sensitivity of soaked seeds to injury by x-radiation. In the experiments to be described, however, it was found that increased height over that of the controls was secured when the dry seeds were given certain doses of medium intensity. Conclusions, therefore, were not published until three series of experiments were concluded. The first and third of these were begun during successive Januaries and the second one in May.

Methods

In the first experiment, seeds of lot I comprising 5 groups each of 100 dry Marquis wheat grains were irradiated with doses of 1000, 5000, 10,000, 20,000, 40,000, and 60,000 r-units, respectively. During treatment, all seeds were in Petri dishes, covered with cellophane. The seeds were then soaked for 18 hours, left in germinators for 12 hours, and later planted in plots in the University greenhouse. The 5 groups of lot II were similarly treated except that the period of soaking and sprouting in the germinator preceded irradiation.

Observations were made daily of the number of seedlings which showed above ground. By the sixth day after irradiation, seedlings were up in all groups of both lots, those receiving the heaviest doses appearing in diminished numbers. Beginning the tenth day after treatments and continuing weekly for four consecutive weeks and thereafter biweekly until maturity, records were taken of the height of all plants.

Results

SURVIVAL OF PLANTS FROM DRY AND SOAKED IRRADIATED SEEDS

A comparison of the number of seedlings living for a period of five or six weeks after irradiation indicates that doses of 1000, 5000 and 10,000 r-units given to the dry grains in no way affected their survival; 20,000 r-units reduced the number more than half, while heavier doses caused death of all seedlings within a period of 2.5 weeks. Seeds soaked before irradiation were much more sensitive to injury than the dry ones; this is shown by the fact that 5000 r-units reduced survival to approximately 5 per cent. of the total, and heavier doses caused death within 3 weeks. In the second and third experiments the procedure followed was the same as for the first, except that those doses which had proved lethal to all seedlings in a group were omitted.

Percentage of seedling survival in all three experiments for a period of 6 weeks is shown in table I and by the curves of figure 1. Data used in the

TABLE I

PERCENTAGE OF SURVIVAL FOR FIRST SIX WEEKS OF PLANTS FROM IRRADIATED SEEDS

Dosage	Seeds irradiated dry Weeks after irradiation			Seeds irradiated after soaking Weeks after irradiation		
	2	4	6	2	4	6
	%	%	%	%	%	%
Control						
I	86.0	86.0	85.0	87.0	91.0†	90.0
II	88.0	81.0	84.0†	87.0	82.0	79.0
III	95.0	96.0	96.0	100.0	100.0	100.0
Av. for 3 exp.	89.7	87.7	88.3	91.3	91.0	89.7
1,000 r-units						
I	88.0	95.0†	95.0	88.0	91.0†	91.0
II	83.0	80.0	80.0	84.0	81.0	77.0
III	97.0	95.0	96.0†	94.0	96.0	95.0
Av. for 3 exp.	89.3	90.0	90.3	88.7	89.3	87.7
5,000 r-units						
I	83.0	91.0†	95.0†	72.0	23.0	8.0
II	68.0	66.0	63.0	.....	.....	.....
III	97.0	97.0	97.0	55.0	27.0	5.0
Av. for 3 exp.	82.7	84.7	85.0	63.5*	25.0*	6.5*
10,000 r-units						
I	87.0	93.0†	89.0	39.0	.....	.....
II	89.0	88.0	85.0	.....	.....	.....
III	96.0	95.0	95.0	.....	.....	.....
Av. for 3 exp.	90.7	92.0	89.7	.....	.....	.....
20,000 r-units						
I	71.0	39.0	24.0	47.0	.....	.....
II	70.0	68.0	66.0	.....	.....	.....
III	88.0	76.0	70.0	.....	.....	.....
Av. for 3 exp.	76.3	61.0	53.3	.....	.....	.....
40,000 r-units						
I	53.0	.....	.....	25.0	.....	.....
60,000 r-units						
I	41.0	.....	.....	15.0	.....	.....

* Average for first and third experiments.
† Increased number apparently due to delayed germination and growth.

chart construction are the average percentages obtained in all three experiments. In plants from the groups irradiated dry with doses up to 10,000 r-units there is little difference between experimental plants and controls. Percentage of survival was considerably reduced in plants from seeds receiving 20,000 r-units, while those with heavier doses lived not more than 2.5 weeks. During the six-weeks' period of observation injurious effects of the rays on soaked seeds were more apparent than on dry ones given the same

dose; although for groups of soaked seeds receiving as low a dose as 1000 r-units, survival was only slightly less than for the controls. The percentage of plants living from the 5000 r-units groups of soaked seeds was very low indeed, and not any of those receiving the still heavier doses lived longer than 2.5 weeks. Even during that period, they made practically no growth after pushing through the soil.

Comparative growth at end of six weeks

At the end of six weeks, plants from treated seeds exceeded the controls in at least two of the experiments in the following respects: (a) percentage surviving in the dry groups of 5000 and 10,000 r-units; (b) average height in the dry 1000 r-units group.

In the first two experiments, after the above data were taken, alternate plants were pulled in the following groups: controls, dry groups given the three lighter doses (1000, 5000, and 10,000 r-units), and the 1000 r-units

TABLE II

Average height of seedlings up to heading

Dosage	Seeds irradiated dry — Weeks after irradiation							Seeds irradiated after soaking — Weeks after irradiation						
	2	4	6	8	10	12	14	2	4	6	8	10	12	14
Control														
I	8.4	26.4	34.7	35.7	41.8	51.9	62.3	12.3	25.9	35.3	36.4	40.9	50.9	61.0
II*	14.7	36.0	55.5	71.3	81.0	.....†	.....	16.7	32.1	51.2	66.9	70.8	.....†	.....
III	7.9	22.6	36.1	41.1	49.5	57.4	70.4	9.4	22.4	34.9	42.1	48.7	55.5	66.5
1,000 r-units														
I	8.9	26.6	38.0	40.1	44.4	56.4	71.6	14.8	19.9	34.3	36.2	42.0	51.6	64.6
II	13.5	32.6	55.9	73.7	81.4	.....	.....	7.7	17.2	31.5	49.2	56.4	.....	.....
III	7.4	22.6	38.3	42.2	49.9	58.4	71.8	8.2	20.9	32.1	38.0	44.6	54.1	64.6
5,000 r-units														
I	8.9	27.0	37.7	41.8	46.5	55.3	64.9	1.8	4.6	11.5	13.8	22.9	42.4	47.3
II	13.3	34.0	54.7	75.4	84.8	.....	.....	4.1	18.5	23.1	53.0	69.0	.....	.....
III	6.9	21.6	36.9	42.2	52.1	60.4	70.5	1.1	3.5	23.1	25.6	30.9	43.5	57.4
10,000 r-units														
I	5.8	22.3	32.3	36.3	40.1	49.4	55.1	0.6	Died					
II	14.1	32.0	50.4	75.1	89.1	.....	.....	.....	.....	.....	.....	.....	.....	.....
III	5.8	21.2	35.2	41.2	49.2	56.4	66.2	.....	.....	.....	.....	.....	.....	.....
20,000 r-units	1.3	6.1	14.9	18.8	28.9	42.0	46.1	0.5	Died					
I	9.9	25.8	37.5	57.2	63.4	.....	.....	.....	.....	.....	.....	.....	.....	.....
II	2.3	10.4	20.8	30.0	39.2	48.9	60.2	.....	.....	.....	.....	.....	.....	.....
40,000 r-units														
I	0.5	Died						0.4	Died					
60,000 r-units														
I	0.4	Died						0.5	Died					

* Experiment II begun in May, hence growth was more rapid than that in I and III which were begun in January.

† Experiment concluded.

group of soaked grains. The number of tillers produced in the 1000 and 5000 r-units groups of the dry grains and the 1000 r-units group of the soaked grains exceeded those of the controls. The green weight of the pulled plants in the 5000 r-units dry group was also greater than that in the controls.

Comparative growth up to heading stage

The comparative effects of different doses given the dry grains will first be considered, then a similar comparison for the soaked ones will be made. Table II gives for all three experiments the biweekly measurements which

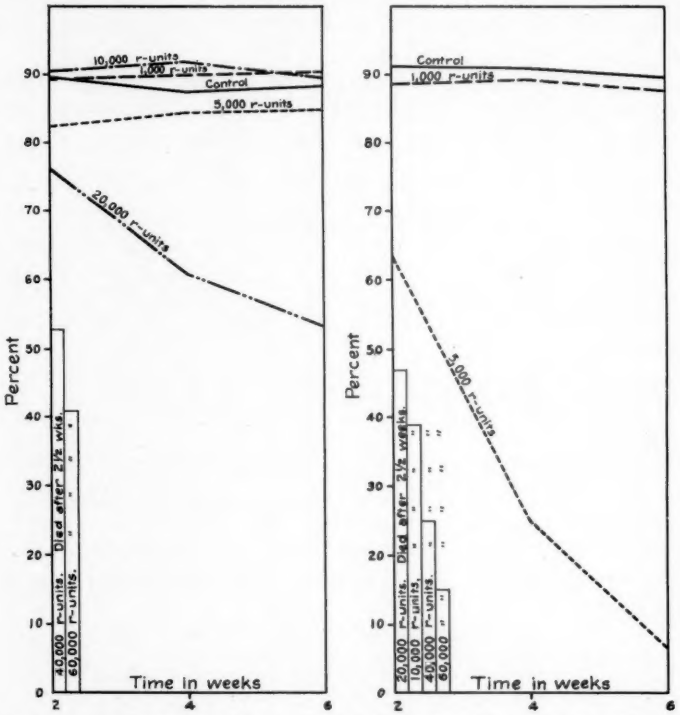

Fig. 1a. Left, survival for first 6 weeks of plants from dry irradiated seeds. Results for control and 4 lighter doses are averages from 3 experiments; others from first experiment only.

1b. Right, survival for first 6 weeks of plants from soaked irradiated seeds. Results for control and 2 lighter doses are averages from 3 experiments; others are from first experiment only.

were taken until the plants began to head out; measurements were then discontinued until maturity, when complete records were made. Plants of the

second experiment, which was begun in May, grew much more rapidly and matured two weeks earlier than those of the first and third experiments which were treated in January. Graphs were made to show heights attained at biweekly periods for all groups in the three experiments. The most representative curves were those plotted from the third experiment; they are here reproduced (fig. 2). The average height from the dry groups receiving 1000

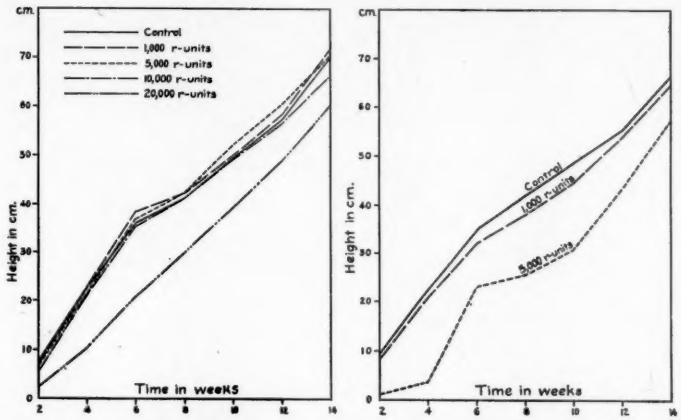

FIG. 2a. Left, height of seedlings from dry irradiated seeds until beginning of heading. Data from experiment III.

2b. Right, height of seedlings from soaked irradiated seeds until beginning of heading. Data from experiment III.

and 5000 r-units in all three experiments exceeded that of the controls; the height of plants from the 20,000 r-units dry group was considerably less. In the soaked groups, the controls averaged higher in all cases except in one experiment only; in this the 1000 r-units group measured slightly more. A dose of 5000 r-units given to soaked seeds caused considerably lessened height in the seedlings.

A comparison of the effects of the same dose upon dry and soaked seeds as judged by total height is presented graphically in figure 3. This was constructed from the height of plants of series I measured at biweekly periods; the results, however, were much the same in the other two experiments as well. To simplify the graph, only three groups were used in its construction. The vigorous growth made by plants from seeds irradiated dry with 5000 r-units exceeded the growth of the controls. This was true not only for plants of experiment I, which is used in the graph, but for all three experiments. Growth from the soaked grains, given the same dose was very much less. As judged by height of seedlings, the amount of injury to the soaked 5000 r-units group was approximately the same as that to the 20,000 r-units dry

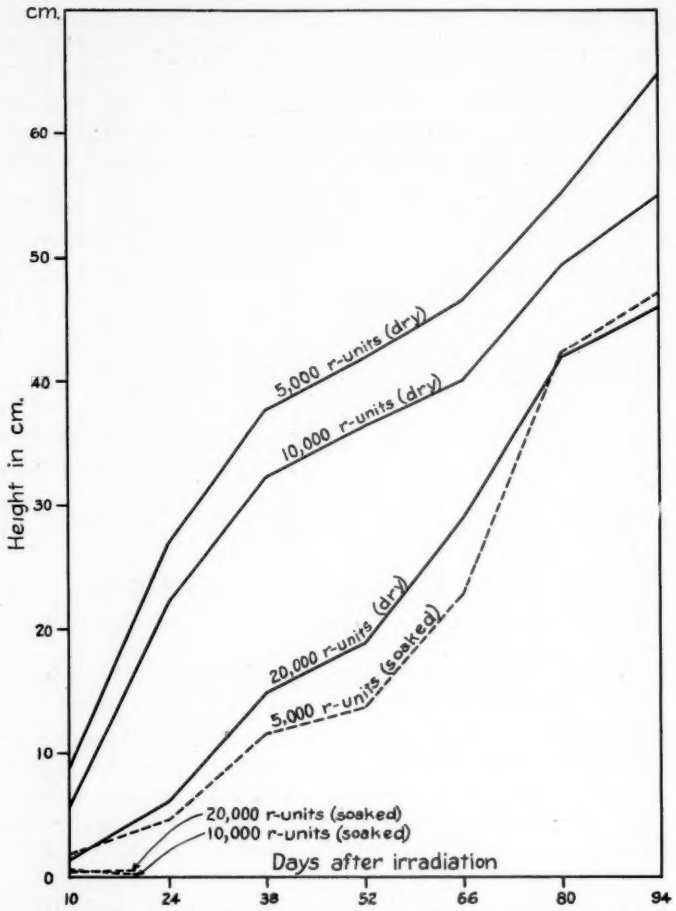

FIG. 3. Comparison of plant growth from irradiated dry and soaked seeds. Data from experiment I.

group. Plants from dry grains given 10,000 and 20,000 r-units show less growth, while the soaked seeds receiving these doses made practically no growth, and were dead by the 20th day after treatment.

GROWTH AT MATURITY

At the conclusion of the experiment, records were taken of (a) the height including head; (b) the height from stem base to the tip of the highest leaf. In addition to these measurements tillers were counted, and the percentage of

tillers producing heads was determined. The green and dry weights were also recorded. From table III which gives data for all three experiments,

TABLE III

GROWTH OF PLANTS AT MATURITY

DOSAGE	NO. OF PLANTS	AV. HEIGHT INCLUDING HEAD	AV. HEIGHT TO TIP OF TALLEST LEAF	AV. NO. OF TILLERS PER PLANT	TILLERS PRODUCING HEADS	AV. FRESH WEIGHT	AV. AIR-DRY WEIGHT*
Seeds irradiated dry		*cm.*	*cm.*		*%*	*gm.*	*gm.*
Control							
I	42	102.1	92.2	3.2	68.4	16.8	4.8
II	42	81.0	72.1	2.0	72.3	6.9	2.3
III	95	91.9	85.5	2.6	76.4	9.5	.....
1,000 r-units							
I	46	107.8	98.8	3.4	75.3	20.0	7.0
II	42	87.4	73.7	1.8	77.3	6.0	2.2
III	96	94.2	88.3	2.7	62.5	9.0	.....
5,000 r-units							
I	47	107.5	93.8	5.2	76.5	32.2	10.3
II	32	87.4	75.6	2.5	71.2	8.2	2.8
III	96	98.1	86.7	2.8	85.0	8.4	.....
10,000 r-units							
I	49	93.3	80.6	4.6	75.9	23.7	7.6
II	44	89.1	76.2	2.5	81.6	8.2	2.6
III	95	94.4	84.2	2.5	67.2	8.4	.....
20,000 r-units							
I	19	112.6	92.8	5.8	81.1	31.0	10.6
II	33	63.4	62.1	2.0	81.5	4.4	1.5
III	56	84.6	79.6	3.5	69.3	1.1	.....
Seeds irradiated after soaking							
Control							
I	47	106.6	92.0	2.5	75.4	14.1	4.5
II	42	70.8	65.9	1.8	78.9	5.2	1.8
III	100	91.7	82.0	3.0	63.2	9.4	.....
1,000 r-units							
I	44	99.3	91.4	3.0	65.6	14.3	4.6
II	35	56.4	60.8	3.2	44.6	5.9	1.4
III	91	98.8	86.7	2.6	69.3	9.5	.....
5,000 r-units							
I	3	91.7	79.0	2.7	75.0	13.3	4.2
II	1	47.0	69.0	9.0	11.0	10.5	3.1
III	4	93.6	81.4	9.2	78.4	47.9	.....

* Owing to an accident, accurate weights were not obtained for experiment III.

the percentage increase over the control was calculated for the first three items listed above for the dry groups. These are presented graphically in figure 4.

Dry-seeded groups receiving 1000 or 5000 r-units in all three experiments exceeded the controls in average height of plants including head, as well as in

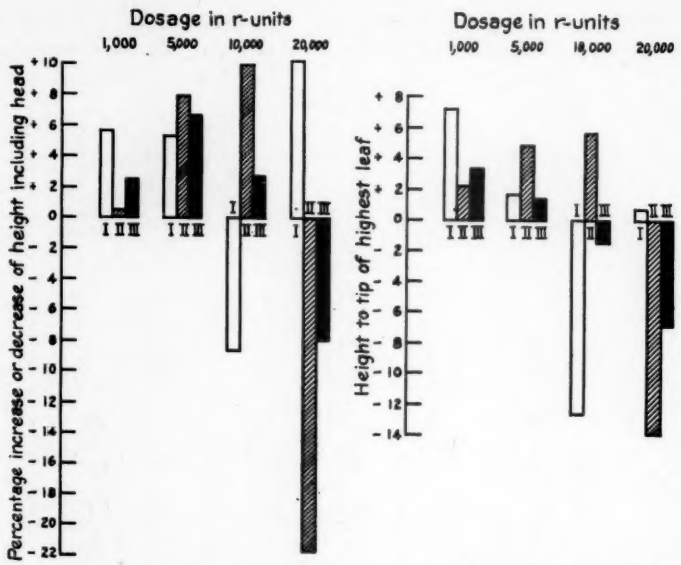

FIG. 4. Percentage increase or decrease over the controls of plants from irradiated dry seeds.

average height to the tip of the highest leaf. In the 10,000 r-units group, in two out of three experiments, the height exceeded that of the controls; this occurred in but one experiment with the 20,000 r-units group.

Since increase in branching is one of the usual results obtained by x-raying plants, it is not surprising to find more tillering in every group surviving the given dose. Figure 5 gives the percentage increase in tillering over the controls. A very considerable increase in the dry groups given 5000 r-units and 10,000 r-units is shown. As pointed out earlier in this paper, the number of plants in the 20,000 r-units group was much reduced, hence the increased space in the plot available because of fewer plants was perhaps a factor in causing increased tillering. Few of the soaked group exceeded the controls in any respect at maturity except in tillering. In the first two experiments, groups receiving 1000 and 5000 r-units respectively showed a great increase in tillering over controls (table III).

Table V shows all the various respects in which at maturity the performance of plants from the irradiated grains exceeded that made by the controls. Roman numerals are used to indicate the experiment in which the growth of experimental plants was greater than that of the controls. There is complete agreement in the results of the three experiments regarding the increase over the control in height and tillering in the 1000 and 5000 r-units

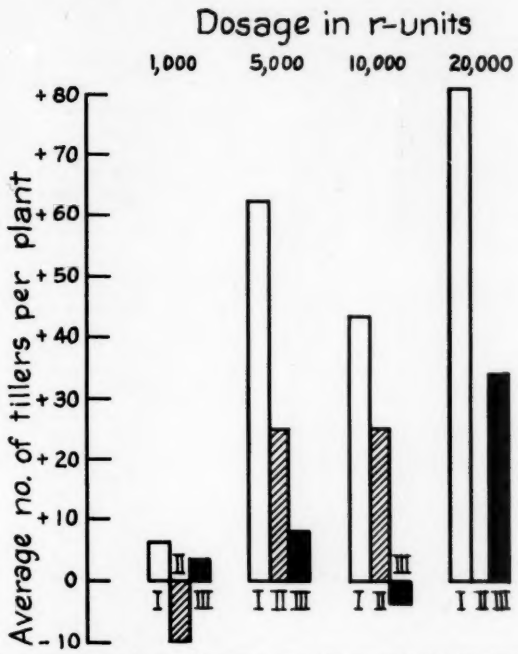

FIG. 5. Percentage increase or decrease over the control of tillering in plants from irradiated dry seeds.

group. This increase, however, does not result in increased green or dry weights in every case, notably in the third experiment. Hence although doses of 1000, and particularly 5000 r-units, apparently increase height and tillering, disagreement as to increase in green and dry weight negatives any claim that these doses cause a stimulation which results in actual increase of plant materials.

Discussion

The procedure followed in these experiments with regard to length of the soaking period for the seeds and the heaviest dose employed, is similar to that used by SUGIURA (3) in a study in which he determined the effects of x-rays on the vitamin B content of wheat seedlings. SUGIURA found that the height of 20-day seedlings from seeds irradiated wet was twice as great as from those irradiated dry, hence he concludes that "wheat seeds in dry, dormant state are more sensitive to x-rays than those in wet physiological state as regards growth of plants after irradiation when doses over 60,000 r-units are given."

TABLE IV

PERCENTAGE INCREASE OR DECREASE OF PLANTS FROM IRRADIATED SEEDS OVER CONTROL

DOSAGE	AV. HEIGHT INCLUDING HEAD	AV. HEIGHT TO TIP OF TALLEST LEAF	AV. NO. OF TILLERS PER PLANT	TILLERS PRODUCING HEADS	AV. FRESH WEIGHT	AV. AIR-DRY WEIGHT*
Seeds irradiated dry	%	%	%	%	%	%
1,000 r-units						
I	+5.6	+7.2	+6.2	+10.1	+19.0	+45.8
II	+0.5	+2.2	−10.0	+6.9	−13.0	−4.3
III	+2.5	+3.3	+3.8	−18.2	−5.3	.....
5,000 r-units						
I	+5.3	+1.7	+62.5	+11.8	+91.7	+114.6
II	+7.9	+4.8	+25.0	−1.5	+8.8	+21.7
III	+6.7	+1.4	+7.9	+14.8	+11.6	.....
10,000 r-units						
I	−8.6	−12.6	+43.7	+11.0	+41.1	+62.5
II	+10.0	+5.7	+25.0	+12.9	+18.8	+13.0
III	+2.7	−1.5	−3.8	−12.0	−11.6	.....
20,000 r-units						
I	+10.3	+0.7	+81.2	+18.6	+84.5	+120.8
II	−21.7	−13.9	0.0	+12.7	−36.2	−34.8
III	−7.9	−6.9	+34.6	−9.3	−88.4	.....
Seeds irradiated after soaking						
1,000 r-units						
I	−6.8	−0.7	+20.0	−13.0	+1.4	+2.2
II	−20.3	−7.0	−77.8	−43.5	+13.5	−22.2
III	+7.7	+5.7	−13.3	+9.7	+1.1	.....
5,000 r-units						
I	−14.0	−14.1	+8.0	−0.5	−5.7	−6.7
II	−33.6	+4.7	+400.0	−85.9	+101.9	+72.2
III	+2.1	−0.7	+206.7	+24.1	+388.3	.....

* Owing to an accident, accurate dry weights were not obtained for experiment III.

Experimenters are agreed that very heavy irradiation of seeds does not prevent germination and some growth. However, practically all have found that heavier doses are required to inhibit growth of dry seeds than soaked ones. According to the experiments described in this paper, doses of 40,000 r-units for the dry seeds and 10,000 r-units for the soaked ones cause cessation of growth after sprouts less than 1 cm. in height have appeared. The writer, therefore, agrees with HENSHAW and FRANCIS (2) that growing material is more strongly affected than non-growing by equal doses of radiation given in the same way.

Summary

Results upon growth of wheat plants are presented from three sets of experiments with dry and soaked seeds exposed to x-ray doses ranging from

TABLE V

PLANTS FROM IRRADIATED GROUPS WHICH EXCEEDED CONTROLS AT MATURITY
(ROMAN NUMERALS REFER TO THE NUMBER OF THE EXPERIMENT)

DOSAGE	AV. HEIGHT INCLUDING HEAD	AV. HEIGHT TO TIP OF TALLEST LEAF	AV. NO. OF TILLERS PER PLANT	PERCENTAGE OF TILLERS PRODUCING HEADS	AV. FRESH WEIGHT	AV. AIR-DRY WEIGHT
Seeds irradiated dry						
1,000 r-units	I II III	I II III	I III	I II	I	I
5,000 r-units	I II III	I II III	I II III	I	I II	I II
10,000 r-units	II III	II	I II	I II	I II	I II
20,000 r-units	I	I	I III	I II	I	I
Seeds irradiated after soaking						
1,000 r-units	III	III	I II	III	I II III	I
5,000 r-units*	III	II	I II III	III	II III	II

* Survival so low that results are not comparable.

1000 to 60,000 r-units. Comparisons are made with controls kept under the same environmental conditions.

1. The percentage of seedlings surviving in the groups treated dry with 1000, 5000 and 10,000 r-units respectively was as high as in the control. A dose of 20,000 r-units, however, reduced the number to less than half; heavier doses applied to dry seeds caused the death, within 3 weeks, of all seedlings. Seeds soaked before irradiation were much more sensitive to injury than dry ones; the seedlings from grains treated with doses heavier than 5000 r-units all died within 3 weeks.

2. Tillers on 6-week-old seedlings from irradiated dry grains were more numerous in 1000 and 5000 r-units groups than in the controls. At maturity, with these same doses, the tillering was increased and also the height, including head, and the distance to the tip of the highest leaf. In the 5000 r-units group, in two of the experiments, the green weight of the plants exceeded that of the controls.

3. Dry grains treated with 10,000 r-units in two out of three experiments increased average height, tillering, percentage of tillers bearing heads, and green and dry weight.

4. Seedlings from soaked grains made less growth in all respects than did the controls, except that the 1000 r-units group tillered more than the controls in two of the experiments, and in the 5000 r-units group in all experiments tillering was considerably increased.

Acknowledgment is made to the Committee on the Effects of Radiation upon Living Organisms of the National Research Council for assistance in

making this study, and to the Physics Department of the University of Colorado for the use of the x-ray machine. Thanks are extended to Professor FRANCIS RAMALEY for helpful criticism of the manuscript.

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GREENHOUSE STUDIES OF THE EFFECT OF CLIPPING THE TOPS OF ALFALFA AT VARIOUS HEIGHTS ON THE PRODUCTION OF ROOTS, RESERVE CARBOHYDRATES, AND TOP GROWTH[1]

C. M. HARRISON

(WITH FOUR FIGURES)

Alfalfa is frequently used as a pasture crop in the state of Michigan, such use being particularly advantageous during the hot summer period. Grazing of livestock on alfalfa generally removes the upper portions of the top growth of the plant first, the grazing being practically uniform over the entire area. This makes it possible to remove the livestock from an alfalfa field before all of the top growth is consumed, or to stock the area so that the basal portions of the shoots are rarely eaten. Management practices which permit prolonged close grazing frequently result in considerable damage to the stands.

In an attempt to determine the amount of top growth necessary to keep alfalfa plants from being weakened, or the reserves of stored food from being depleted, cultures of Hardigan alfalfa plants were set up in the greenhouse at Michigan State College in East Lansing on October 9, 1936. The cultures were grown in quartz sand in 10-inch clay pots from seedling plants collected in the field and transplanted to pots. Eight plants were placed in each pot, and each treatment was replicated five times. All cultures were supplied with the same nutrient solution.

On March 29, 1937, the plants had reached the blooming stage and three cultures were photographed (fig. 1), harvested, and the green and dry weights of tops and roots determined. These three cultures were harvested before any cutting treatments were begun and will be known as "initial" checks. After the roots were washed free of sand a small segment from each of 15 roots was placed in a fixing solution of formalin alcohol to be preserved for histological study of stored starch. The cultures remaining were grouped into lots of 5 and cut weekly from March 31st until May 26th, when the experiment was discontinued. Five cultures were left uncut throughout the duration of the experiment and were harvested when the experiment was completed. These 5 cultures will be known as "final" checks. In the case of the final checks the alfalfa in the greenhouse had reached a condition comparable to that in the field when alfalfa is ordinarily cut for hay. The yield of green and dry weight of tops was determined for each culture within each cutting treatment. The cutting treatments were as follows:

[1] Contribution from the Section of Farm Crops, Michigan Agricultural Experiment Station, East Lansing, Michigan. Journal Article no. 320 (new series).

1. Cut to 1 inch weekly.
2. Cut down gradually for 4 successive weeks until a 2-inch height was attained, after which this 2-inch height was maintained.
3. Cut to 6 inches weekly.
4. No cutting, or "final" checks.

The production in grams of green and dry material from the cultures is recorded in table I.

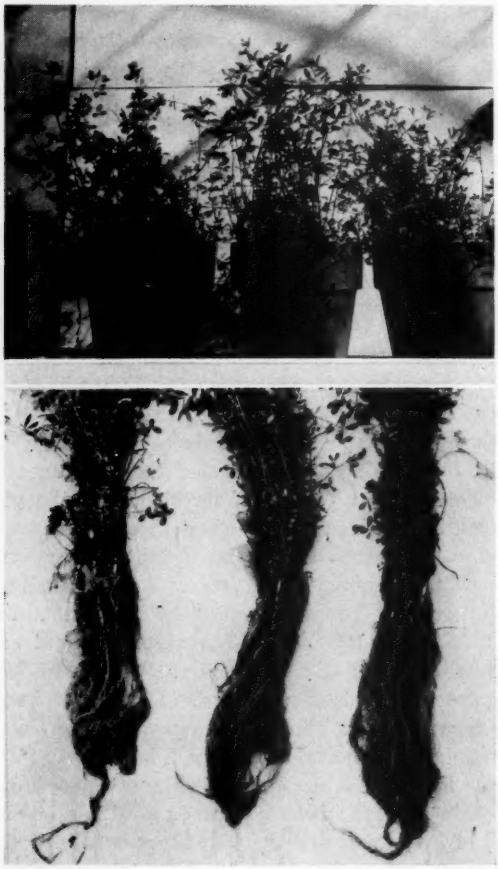

FIG. 1. Cultures of alfalfa plants before cutting was begun on 3/29/37. Above, before washing sand from roots. Below, after sand was washed from roots.

TABLE I

PRODUCTION OF ALFALFA IN GREEN AND DRY WEIGHTS PER POT WHEN CUT AT DIFFERENT HEIGHTS

TREATMENT	DATE	3/31		4/7		4/14		4/21		4/28		5/5	
	POT NO.	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
	1	60.0	14.0	5.7	1.05	9.2	1.45	8.8	1.54	4.0	0.84	3.5	0.75
	2	60.7	16.8	14.0	1.97	10.0	1.51	9.0	1.25	4.5	0.73	3.8	0.66
Cut to 1 inch	3	69.0	18.0	10.0	1.45	12.0	1.92	6.8	0.93	3.8	0.63	2.5	0.49
	4	57.8	13.8	6.0	1.05	10.0	1.57	7.5	1.19	3.0	0.58	2.8	0.54
	5	69.5	16.0	9.2	1.68	13.2	2.37	10.0	1.66	6.0	1.31	5.5	1.06
Total		317.0	78.6	44.9	7.20	54.4	8.82	42.1	6.57	21.3	4.09	18.1	3.50
	1	13.7	3.5	21.4	4.43	32.0	6.31	56.0	12.0	3.0	0.53	15.5	2.61
	2	14.5	2.7	18.5	3.61	29.0	5.36	47.5	9.5	3.0	0.40	11.5	1.91
Cut to 2 inches gradually	3	13.0	2.2	22.0	4.02	23.0	3.99	41.5	8.4	4.0	0.60	12.0	2.00
	4	12.5	2.5	24.0	4.65	29.8	5.47	51.5	9.5	4.8	0.77	14.0	2.26
	5	20.0	4.6	24.5	5.03	22.0	4.28	36.0	7.2	4.8	0.75	13.0	2.14
Total		73.7	15.5	110.4	21.74	135.8	25.14	232.5	46.6	19.6	3.05	66.0	10.92
	1	30.3	7.0	2.5	0.49	11.8	2.09	13.0	2.26	8.0	1.51	9.5	2.03
	2	37.5	8.0	6.8	1.35	11.6	1.93	15.0	2.45	16.0	2.68	12.5	2.49
Cut to 6 inches	3	32.7	7.5	7.8	1.44	7.0	1.15	15.0	2.51	8.0	1.48	17.0	3.48
	4	37.0	8.9	8.6	1.57	9.2	1.54	16.8	2.70	7.0	1.28	11.0	2.17
	5	42.5	10.0	10.8	2.30	12.0	2.03	18.0	2.51	8.0	1.45	13.8	2.89
Total		180.0	41.4	36.5	7.15	51.6	8.74	77.8	12.43	47.0	8.40	64.8	13.06
Initial checks before cutting was begun 3/29	1	95.0	20.0										
	2	118.2	26.5										
	3	100.0	21.5										
Final checks at last harvest date, 5/26; no cutting	1	266.0	58.0										
	2	195.0	47.5										
	3	197.0	47.0										
	4	171.0	46.5										
	5	114.0	26.5										

TABLE I—(*Continued*)

Treatment	Date	5/12		5/19		Final harvest date 5/26		Total		Average per pot		Dry matter
	Pot no.	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%
	1	3.5	0.61	2.5	0.36	13.5	3.0	110.7	23.6			21.3
	2	2.8	0.45	2.0	0.28	13.0	2.8	119.8	26.5			22.1
Cut to 1 inch	3	2.0	0.36	1.8	0.28	12.0	2.0	119.9	26.1			21.7
	4	2.4	0.38	2.5	0.31	11.0	2.5	103.0	21.5			20.9
	5	6.0	1.19	5.5	0.94	27.0	5.2	151.9	31.4			20.7
Total		16.7	2.99	14.3	2.17	76.5	15.5	605.3	129.1	121.0	25.8	21.3
	1	10.0	1.68	13.5	2.16	60.0	12.5	225.1	45.7			20.3
	2	15.0	2.38	12.0	1.81	66.0	15.5	217.0	43.2			19.9
Cut to 2 inches gradually	3	11.0	1.72	10.5	1.66	54.0	12.5	191.0	37.1			19.4
	4	15.0	2.40	13.0	2.00	71.0	16.3	235.6	45.9			19.5
	5	11.0	1.73	11.0	1.72	55.0	11.0	197.3	38.5			19.5
Total		62.0	9.91	60.0	9.35	306.0	67.8	1066.0	210.4	213.2	42.1	19.7
	1	11.0	2.27	15.0	3.04	158.0	33.0	259.1	53.7			20.7
	2	11.0	1.92	18.0	3.37	157.0	28.0	285.4	52.2			18.3
Cut to 6 inches	3	9.0	1.85	14.0	2.72	151.0	34.0	261.5	56.1			21.5
	4	9.5	1.78	11.5	2.26	156.0	34.5	265.9	56.7			21.3
	5	9.0	1.78	13.0	2.54	193.0	35.5	320.1	61.0			19.1
Total		49.0	9.60	71.5	13.93	815.0	165.0	1392.0	279.7	278.4	55.9	20.1
Initial checks before cutting was begun 3/29	1 2 3							313.2	68.0	104.4	22.7	21.7
Final checks at last harvest date, 5/26; no cutting	1 2 3 4 5							943.0	225.5	188.6	45.1	23.9

A study of the table shows that the cultures cut to one inch yielded approximately as much as did those cut to 6 inches for the two weekly periods following the initial cutting. On April 21st, however, three weeks after the initial cutting, the cultures cut at 6 inches produced more new growth above the point of clipping than did the cultures cut to one inch. This same relation held true until the close of the experiment. On May 19th, one week before the final harvest date, the cultures cut at 6 inches were producing approximately 6 times as much new growth as were the cultures cut to one inch. It is difficult to compare the cultures, cut gradually down to a 2-inch height, with those subjected to the other two cutting treatments. The cultures cut gradually to 2 inches were clipped shorter each week up to the fourth week, when they were finally cut down to two inches. Consequently the production recorded for these cultures was not only "recovery" growth, but also a certain portion of the original top. It will be noted that these cultures produced more dry weight than those cut to one inch continuously except on the 28th of April, which was the date following the last removal down to two inches. Toward the end of the experiment, these cultures were producing less "new growth" above the point of cutting than were those cut to 6 inches, an indication that the reserves were being depleted in the cultures cut at two inches.

After the initial cutting date, growth in the cultures cut to one inch was very meager. The average total production of dry matter per pot in the top growth was only slightly greater than that secured from the initial

TABLE II

AVERAGE PRODUCTION IN DRY WEIGHT PER POT FOR VARIOUS CUTTING TREATMENTS

TREATMENT	TOTAL PRODUCTION OF DRY MATTER INCLUDING FIRST AND LAST CUTTINGS	YIELD IN PERCENTAGE OF INITIAL CHECKS	INCREASE IN PRODUCTION OF DRY MATTER DURING PERIOD OF CUTTING WHEN COMPARED TO INITIAL CHECKS†	PRODUCTION COMPARED TO FINAL CHECKS UNCUT UNTIL MAY 26
	gm.	%	*gm.*	%
Initial checks March 29*	22.7	100	Not cut after March 29	
Final checks on last cutting date 5/26	45.1	198	22.4	100
Cut to 1 inch weekly 3/31 to 5/26	25.8	113	3.1	13.8
Cut to 2 inches gradually	42.1	185	19.4	86.7
Cut to 6 inches weekly 3/31 to 5/29	55.9	246	33.2	148.4

* March 29 was the date when the various cutting treatments were started.
† This represents dry matter removed in various cutting treatments after March 29.

check pots on March 29th, when the cutting treatments were begun. The production of top growth secured from the cultures cut gradually to two inches was approximately twice that of the initial checks and almost equal to the production of the final checks cut only on May 26th. The average total production per pot of the cultures cut to 6 inches was almost three times as much as that of the initial checks, more than double that of the cultures cut to one inch, and approximately 25 per cent. greater than that of the cultures cut to two inches or of the final checks.

Table II summarizes the production of the different cutting treatments.

The percentage of dry matter in the tops was approximately equal for all the cutting treatments.

When the cultures were finally harvested, on May 26th, the sand was washed carefully from the roots, and the green and dry weights of the roots were determined and recorded in table III. Photographs were taken of an individual culture from each treatment before harvesting, and after the

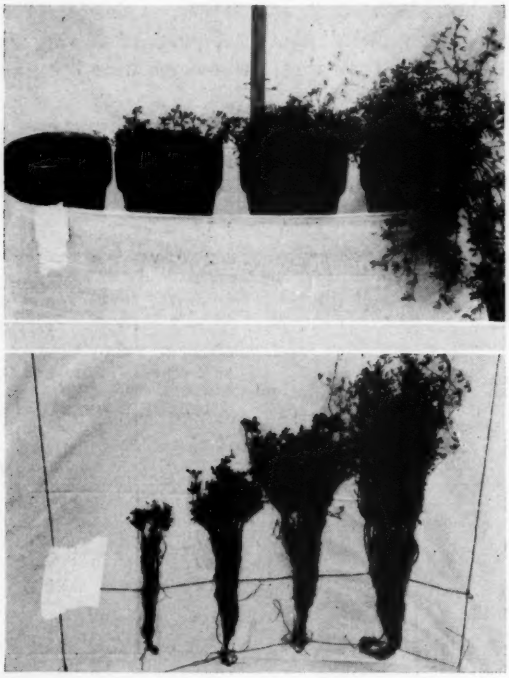

FIG. 2. Alfalfa cultures cut weekly for 8 weeks, before and after washing sand from roots. Left to right, cut to one inch, to two inches gradually, to 6 inches, and uncut check.

TABLE III

GREEN AND DRY WEIGHTS OF ALFALFA ROOTS FROM DIFFERENT CUTTING TREATMENTS

TREATMENT	POT I		POT II		POT III		POT IV		POT V		AVE.		PRODUCTION OF DRY MATTER OF ROOTS COMPARED TO INITIAL CHECK	DRY MATTER	PERCENTAGE OF DRY MATTER IN ROOTS COMPARED TO INITIAL CHECK
	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT			
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%	%	%
Initial check before cutting was begun 3/29	63.8	11.7	90.8	16.0	84.7	17.5					79.8	15.1	100.0	18.9	100.0
Cut to one inch weekly	57.0	9.5	50.0	8.5	33.0	4.5	34.0	5.0	71.0	10.5	49.0	7.6	50.0	15.5	82.0
Cut to two inches gradually	141.0	23.0	107.0	21.0	68.0	14.5	84.0	12.0	126.0	25.5	105.2	19.2	127.0	18.1	95.0
Cut to 6 inches weekly	206.0	49.5	118.0	23.5	172.0	51.0	168.0	39.5	217.0	55.0	176.0	43.7	288.0	24.8	131.0
Final checks no cutting	230.0	63.0	138.0	34.0	131.0	37.5	215.0	66.5	143.0	41.5	171.0	48.5	323.0	28.3	149.0

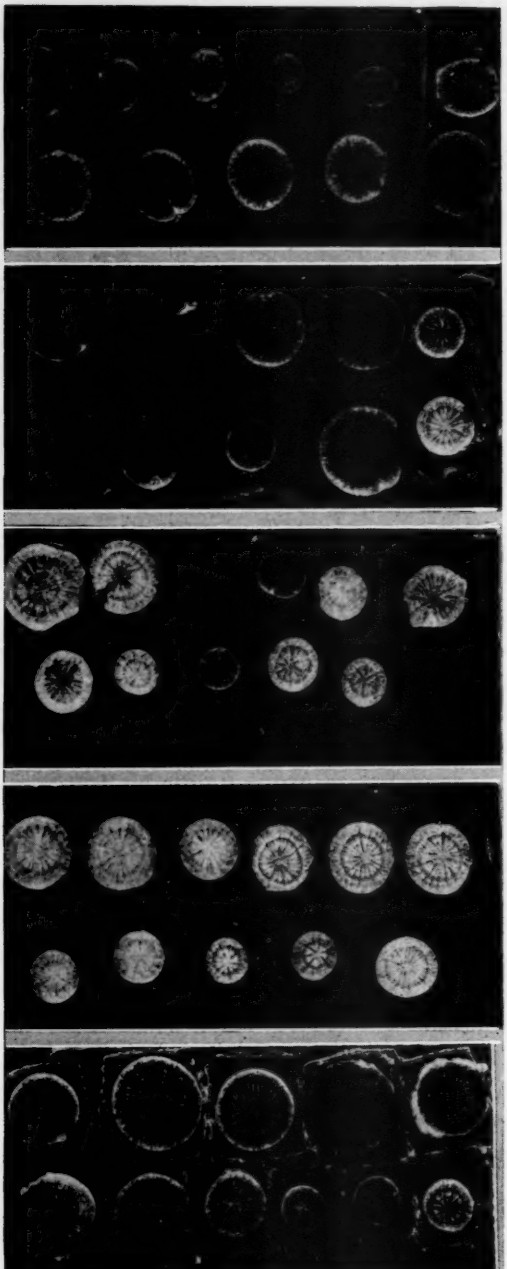

FIG. 3. Photograph of eleven individual alfalfa roots from each of the various cutting treatments showing stored starch. Top to bottom, checks at the beginning of the experiment on 3/29/37; after cutting to 6 inches for 8 consecutive weeks; after cutting to two inches gradually; after cutting to one inch; and checks at the close of the experiment on 5/26/37 (×1½).

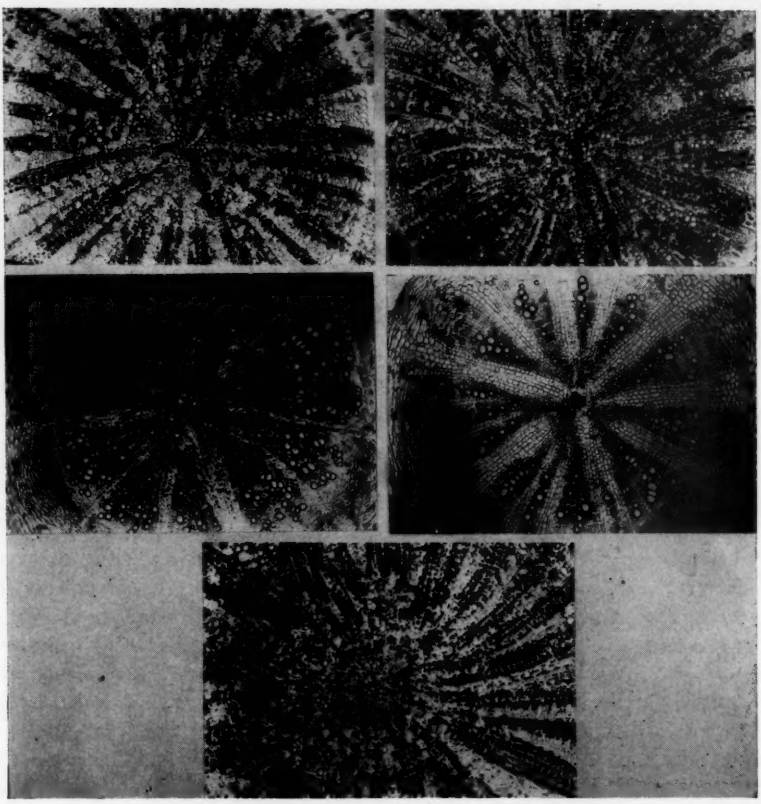

FIG. 4. Photomicrographs of alfalfa roots from the various treatments showing stored starch. Left to right: Top, check on 3/29/37, and after cutting to 6 inches for 8 consecutive weeks; center, after cutting to two inches gradually, and to one inch for the same period; bottom, check at close of experiment on 5/26/37 (×50).

sand was washed from the roots, in order to note the quantity of roots present (fig. 2). A small segment of approximately 15 roots from each treatment was fixed in formalin alcohol for histological study.

It will be noted from a study of table III that the average dry weight of the roots from the cultures cut to one inch continuously was approximately half as great as the weight of roots harvested before cutting was begun. The dry weight of roots from the cultures cut gradually to two inches was only slightly greater than the weight of the roots of the original checks, while the roots of the cultures cut to 6 inches, and those of the final checks, had a dry weight practically three times as great as that of the initial

checks. It seems that cutting at one inch not only prevented the growth of new root tissue and accumulation of reserve materials, but also brought about a loss of root tissue present when cutting was begun. Roots of the cultures cut gradually to two inches increased only slightly in dry weight over that of the initial checks, while the 6-inch cutting treatment allowed the production of root growth almost as great as that of the final checks. A comparison of the average total production and the percentage dry matter in the roots of the various treatments is shown in table III. If the percentage of dry matter in the roots before cutting was begun is given the value of 100, the figures in the last column of the table give the comparative values for the other treatments. Substantial reductions may be noted in the case of the tops which were cut at one or two inches. Increases are found in the case of the plants which were uncut or cut at 6 inches.

This variation in percentage of dry matter indicated a difference in the quantity of material stored in the roots from the various treatments. In order to study this condition the roots fixed at the final harvest date, and also those fixed before cutting was begun, were inbedded by the paraffin method and sections made at 15 μ. A section from each of 11 roots from each treatment was mounted on a slide, stained with Gram's iodine solution and photographed. Photomicrographs were likewise made of one root from each treatment, selected as representative of its group. Figures 3 and 4 show the comparative differences in stored starch of the roots from the various treatments. The roots of the plants cut to one inch continuously are seen to have little if any starch remaining, while those from the culture cut gradually to two inches have had their supply reduced (about half of the roots are still quite well supplied, whereas in the other half the starch is practically gone). The roots from the original checks, the final checks, and from the plants cut at 6 inches continuously show an abundant storage of starch.

Discussion

When the top growth of alfalfa plants was removed weekly at specified levels for a period of 8 successive weeks, various responses were noted in the production of new top and root growth and root storage, as compared with the responses of uncut cultures. Marked reductions were found in all respects in cultures cut at one inch; somewhat less prominent reductions occurred in cultures cut at two inches. Cutting at 6 inches appeared to interfere only slightly with the functioning of the plant in the production of top and root growth and stored material as compared with the uncut cultures.

In pasturing alfalfa, close removal of top growth is often allowed. This practice brings about a reduction in the production of dry matter by the plants, as well as the depletion of any previously stored reserve materials.

Unless given an opportunity to grow and replenish these reserves the plants may die. Frequently the plants die during the growing season, although the hazards to the stand are particularly severe if stored food has been depleted just previous to freezing weather. Reduction of the stand under close grazing actually occurs in field practice, whereas very little difficulty is encountered if the alfalfa is allowed to retain from 6 to 10 inches of top growth during the grazing season. It may be assumed that the close and frequent removal of the top growth of alfalfa in the greenhouse tests is an indication of the conditions brought about by too close grazing in the field and that the maintenance of a reasonable top growth is necessary to keep alfalfa in fairly good condition. Grazing to one or two inches may so deplete the materials stored in the roots that the plants will be weakened and, unless given a chance to replenish their food reserves, will die from carbohydrate starvation or be more subject to winter injury.

In many of the schemes of pasture management which have been proposed, there has been a pronounced tendency to insist that plants suitable for grazing must be so constituted physiologically that they will "stand abuse,"—in particular, close grazing. But plants which survive best under close grazing are not always most productive in a given environment. If the results reported here are reasonably indicative of field performance, it appears entirely feasible to so manage the grazing of alfalfa as to secure top growth production and storage of food reserves in the roots comparable to that obtained when the crop is well managed for hay.

In the event of the general application of this principle to other plants, it seems reasonable to venture that the development of different schemes of pasture management, suited to the physiology of existing plants of superior productive merit, may be at least as promising a means of pasture improvement as the selection of special types of plants which may withstand the "abuse" of a fixed system of management based on close grazing.

Summary

1. Alfalfa cultures grown in the greenhouse were cut to one inch, two inches gradually, and to six inches continuously for eight successive weeks from March 31st to May 26th.

2. Subsequent growth of tops was materially lessened in cultures which were cut to one inch, in comparison to those cut gradually to two inches and those cut to six inches.

3. The percentage of dry matter of top growth from the various treatments was approximately equal.

4. Cultures of alfalfa plants which were cut to one inch weekly for eight consecutive weeks had roots at the close of the experiment which contained only 50 per cent. as much dry matter as they did when the cutting

was begun, and approximately 14 per cent. as much as was found in the roots of plants not cut until the close of the experiment. These uncut plants had developed in the greenhouse to a maturity comparable to that of alfalfa in the field when it is commonly cut for hay.

5. In cultures which were cut to two inches gradually, the average dry weight of roots remained approximately equal to that which prevailed when cutting treatments were begun, but they produced only 40 per cent. as much dry matter as the alfalfa harvested only at the close of the experiment.

6. In cultures cut to 6 inches each week for 8 consecutive weeks, the average dry weight of the roots was approximately equal to that of the cultures harvested only at the close of the experiment, was three times as much as that of the checks at the beginning of cutting treatments, and six times as much as that of the cultures cut to one inch.

7. The stored starch in the roots from cultures cut to one inch was entirely depleted, that in the cultures cut gradually to two inches had been partially depleted, whereas the roots sampled at the beginning of the cutting treatments, the roots of cultures cut to 6 inches and the roots of cultures cut only once, at the end of these trials, had an abundant supply of starch.

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EQUIPMENT FOR THE GROWING OF PLANTS AT CONTROLLED TEMPERATURES[1]

E. MARION BROWN

(WITH SEVEN FIGURES)

Introduction

Equipment designed for growing plants under conditions of controlled temperature was installed at the Missouri Experiment Station, Columbia, Missouri, in the fall of 1936. Some features of this apparatus are modifications of temperature control equipment devised and used by other workers. Other features, particularly the use made of standard air-conditioning equipment, are believed to represent new developments in apparatus for the growing of plants at controlled temperatures. The description which follows is presented with the idea that other investigators might make use of certain features in the designing of other equipment for the growth of plants in controlled environments.

Description of apparatus

The temperature control equipment described herein consists of 3 growth chambers, the devices necessary for the regulation of temperature in them, and the greenhouse in which they are housed. The greenhouse, 15 feet wide by 28 feet long, is oriented so that its long axis runs east and west. The growth chambers are located along its south side, with a space of approximately 6 feet left between the end compartments and the end walls of the greenhouse, an arrangement which provides equal lighting for all compartments. All three growth chambers operate simultaneously with air and soil temperature independently controlled.

A general view of the greenhouse and the temperature control equipment which it contains is shown in figures 1 and 2.

GROWTH CHAMBERS

Each growth chamber consists of a soil temperature tank and an air chamber. The soil temperature tank occupies all of the compartment, except a 6-inch space between the tank and rear wall, to a height of 3 feet above the floor. The air chamber is that part of the compartment above the soil temperature tank. This space is limited at the top and on the south side by a portion of the roof and south wall of the greenhouse.

The front and side walls of each air chamber, and the doors in the rear,

[1] Contribution of the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Missouri Agricultural Experiment Station, Columbia, Missouri.

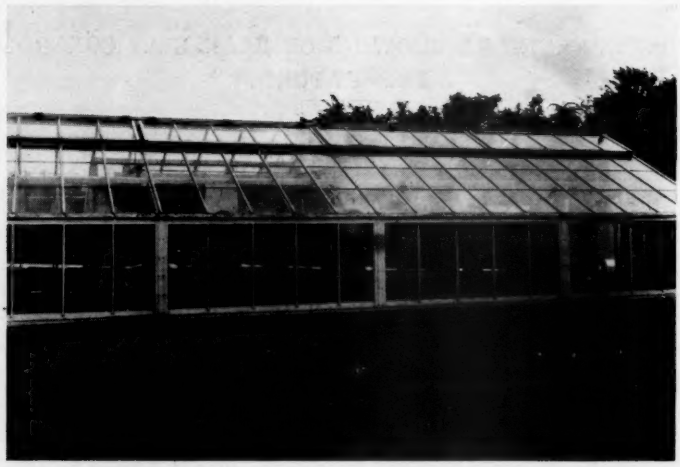

FIG. 1. Greenhouse containing the temperature control units as viewed from north.

are fitted with double panes of glass, separated by a 1-inch air space to reduce heat loss. The roof, however, is covered by a single thickness of standard greenhouse glass, in order to admit light with a minimum of absorption by the glass. The partitions and rear walls of the compartments below the air chamber consist of double walls of masonite, the panels of which are separated

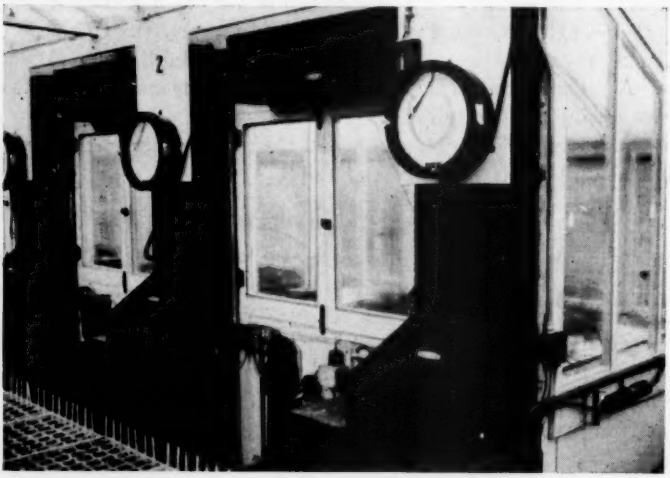

FIG. 2. Interior of greenhouse showing temperature control units.

by a 2-inch air space. The concrete side wall of the greenhouse forms the front wall of each compartment below the air chamber.

The foregoing description, as well as that which follows, will be clarified by reference to the drawings presented (figs. 3 to 6). Dimensions of the greenhouse and growth chambers are shown in figures 5 and 6.

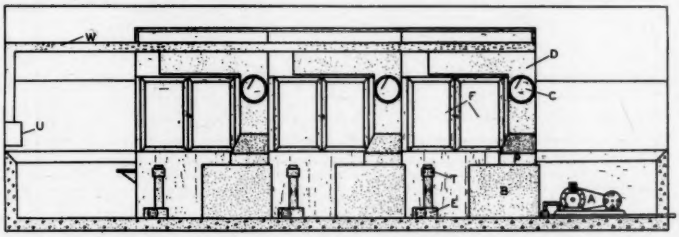

FIG. 3. Longitudinal section of greenhouse showing location and arrangement of temperature control units. A, compressor; B, air cooling unit; C, recording temperature controller; D, air duct; E, rotary pump and motor; F, doors to air chamber; P, air heater; T, immersion heater; U, relays operating heaters; W, electrical conduit.

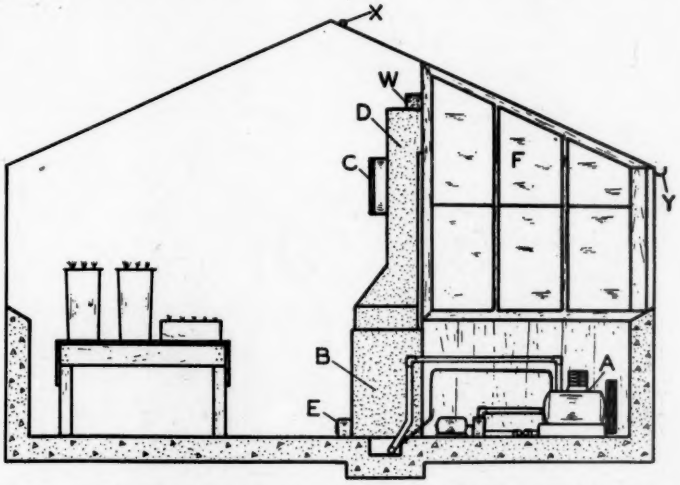

FIG. 4. Cross-section of greenhouse containing temperature control units. A, compressor; B, air cooler; C, recording temperature controller; D, air duct; E, motor operating rotary pump; F, glazed wall of air chamber; W, electrical conduit; X, roof irrigator; Y, gutter.

MAINTAINING CONSTANT AIR TEMPERATURES

The air temperature is held constant by continuously circulating the air through the air chamber and the temperature regulator just outside and back

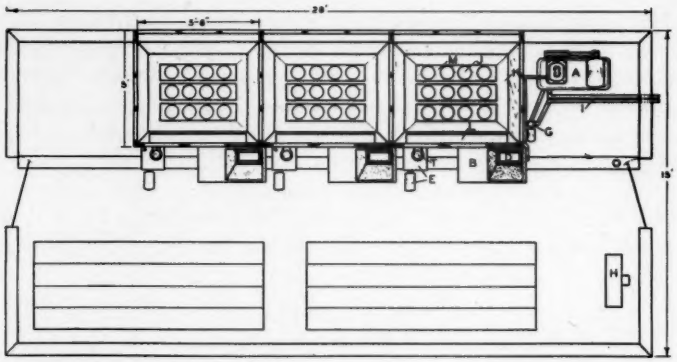

FIG. 5. Floor plan of greenhouse and temperature control units. A, compressor; B, air cooler; D, air duct; E, G, rotary pumps and motors; H, unit heater (steam); I, water pipes to cooling tower; J. soil container; K, soil temperature tank; L, baffle over air outlet; M, sleeve cover.

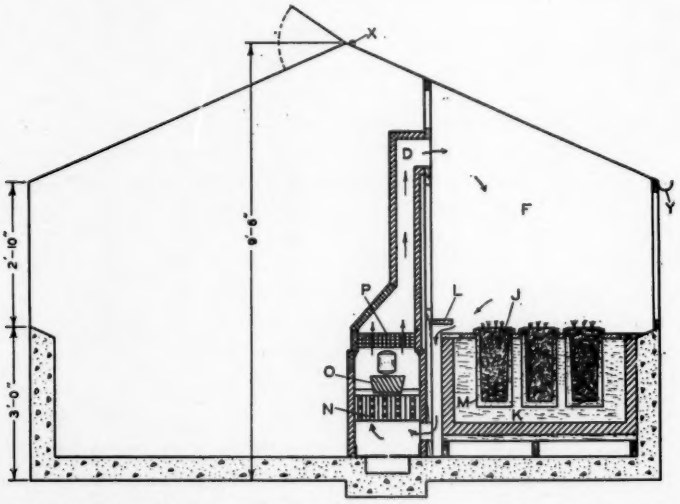

FIG. 6. Cross-section of greenhouse and temperature control unit. D, air duct; F, air chamber; J, soil container; K, soil temperature tank; L, baffle over air outlet; M, sleeve; N, cooling coils; O, fan; P, electric heater.

of each compartment (B and P, figs. 3 to 5). The course followed by the circulating air, as well as the essential parts of the temperature regulator, are shown in the cross-section of greenhouse and temperature control unit presented in figure 6. The air, withdrawn from the air chamber (F, fig. 6)

under the baffle (L, fig. 6), passes between the soil temperature tank and rear wall of the compartment into the lower part of the temperature regulator, through a short air duct. As the air passes through the temperature regulator it is either heated or cooled, depending on its temperature as it leaves the air chamber, relative to that which the controls are set to maintain. The heated or cooled air is returned to the air chamber through the air duct (D, fig. 6), the outlet of which extends nearly the full width of the air chamber near the top. This outlet is provided with curved deflectors placed to give a uniform distribution of the inflowing air throughout the air chamber.

The cooling unit of the air temperature regulator is a Frigidaire room cooler, Model C–100–B. It consists of a cabinet containing refrigeration coils provided with fins to increase their cooling surface (N, fig. 6) and a fan which circulates the air (O, fig. 6). The heating unit is a Chromalox Air Blast Heater, Model CAB–5, mounted in the air duct just above the cooling unit. Each heater is divided into two equal 1500-watt, 220-volt, 3-phase circuits, either of which can be switched on independently of the other, to increase or decrease the amount of heat supplied to the air. The heaters are equipped with thermo cut-outs to break the circuit in case of overheating. The entire air temperature regulating unit and air duct leading from it to the air chamber are covered by a one-inch layer of cork for insulation.

In order to reduce the absorption of heat by the air chamber and lighten the cooling load during warm, clear days, a sheet of water is run over the south slope of the greenhouse roof. A ¾-inch copper pipe with small nozzles inserted at 6-inch intervals extends the full length of the roof just below the ridge (X, figs. 4 and 6). By this means three streams of water, each approximately 1/20 inch in diameter, are directed against each 16-inch pane of glass at an angle which spreads the water in a thin sheet over the full width of the sloping glass roof. The water is drained away by a gutter along the south eaves (Y, figs. 4 and 6). Tap water, having a summer temperature of 60° to 70° Fahrenheit is used for this purpose and is not recirculated.

Soil temperature tank

The soil temperature tank (K, fig. 6) consists of an iron tank, insulated by a 2-inch layer of cork which is in turn covered by an outer sheath of lighter material. Built into each tank are three iron sleeves (M, fig. 6), each 10 inches wide, 40 inches long, and 20 inches deep. These sleeves are welded to the top of the tank so that only the sleeves open into the air chamber. Over each sleeve there is a detachable cover made of 2-inch cypress in which four equally spaced circular openings have been cut to hold the soil containers.

The tank is filled with water or some non-freezing solution, depending on the temperature which is to be maintained. The tank solution is prevented from coming in contact with the soil containers by the metal sleeves in which

the containers are suspended. However, the sleeves themselves can be filled with water or some other solution in order to facilitate the transference of heat between soil and tank.

The pots holding the soil in which the experimental plants are grown are made of 24-gauge galvanized iron soldered at the seams to make them watertight. They are 8 inches in diameter at the top, 7 inches in diameter at the bottom, and 18 inches deep. Each soil can has a flange made of ½-inch angle iron riveted to its upper rim. This flange not only strengthens the pot, but also supports it when it is suspended in the sleeve of the soil temperature tank, the flange resting on the edge of the opening in the cover.

Each soil container is also provided with a bail which, when turned down, fits snugly against the upper edge of the flange.

In order to maintain the liquid in the soil temperature tanks at a constant temperature, each tank is provided with a cooling coil, a heating unit, and a circulatory system. A perspective view of one of the soil temperature tanks with cover and sleeves removed to show the temperature regulating devices is represented diagrammatically in figure 7.

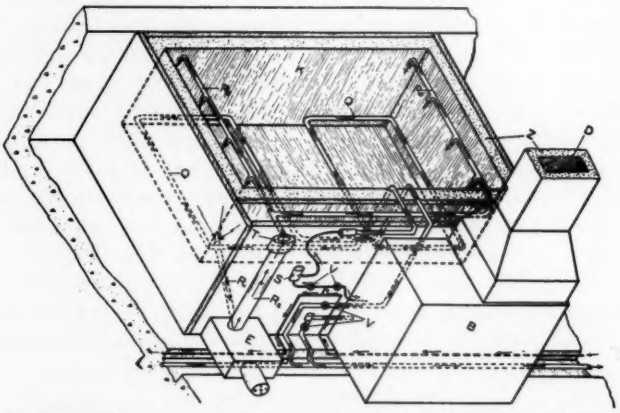

FIG. 7. Perspective view of soil temperature tank with cover and sleeves removed. B, air cooler; D, air duct; E, rotary pump; K, tank; Q, cooling coil; R_1, outlet pipe from tank to pump; R_2, R_3, return pipes from pump to tank; S, solenoid valve; T, immersion heater; V, refrigerant valves; Z, cork insulation.

The cooling coil consists of a 25-foot length of ½-inch copper tubing resting on the bottom of the tank (A, fig. 7). The flow of refrigerant through the coil is regulated automatically by a solenoid valve (S, fig. 7), or manually by opening and closing a gas valve placed in the refrigeration line (V, fig. 7).

A uniform temperature throughout the tank is maintained by constantly circulating the solution. This is accomplished by drawing the liquid from

the bottom of the tank through a pipe (R_1, fig. 7) leading to a motor driven rotary pump (E, fig. 7), and returning it at the top of the tank near each end through 8 spaced outlets in the return pipes (R_3, fig. 7). The pump and return pipe (R_2, fig. 7), which lie outside of the compartment, are protected against rapid heat loss or absorption by cork insulation.

Heat is supplied to the solution by a Chromalox Immersion Heater, similar to Model M–175. It is inserted in a T-joint at the upper end of a vertical 2-inch return pipe (T, fig. 7). This heater, rated at 300 Watts, has a blade 9 inches long and is operated by a 110-volt, single phase circuit.

Refrigeration

All cooling coils are connected to a Frigidaire Model FW6J compressor located inside the greenhouse (A, figs. 2, 4, and 6). The compressor has a water-cooled condenser, through which water is circulated by means of a motor-driven rotary pump (G, fig. 5). The water is cooled by being run through a cooling tower outside of the greenhouse, and is then recirculated through the condenser. A 3-horsepower motor operating on a 220-volt, 3-phase circuit drives the compressor. Freon is the refrigerant.

The compressor motor is switched on and off by means of a pressure switch operated by changes in the pressure of the refrigerant and acting through a relay. In this manner, the opening of any one of the six solenoid valves, each of which controls the flow of refrigerant through a cooling coil, will cause the compressor to operate. When all solenoid valves are closed, the compressor stops. An excessively high pressure caused by the failure of water to flow through the condenser also breaks the circuit to the compressor motor.

The same pressure switch and relay which automatically start and stop the compressor motor also start and stop simultaneously the pump which circulates water through the condenser and the fan in the cooling tower. An additional circuit is provided, however, by which the water-circulating pump can be put into continuous operation independent of the operation of the compressor. This is done in cold weather to prevent freezing in the water pipes outside of the greenhouse. The cooling tower fan can also be put into continuous operation, or turned off.

Control of temperature

Each temperature control unit is equipped with a Brown two-pen recording control thermometer model 6422–830 (C, figs. 3 and 4). These instruments simultaneously record and control the temperature of both the air chamber and soil temperature tank. The thermometer bulbs and capillary tubing are of the mercury expansion type. One bulb of each instrument is

immersed in the solution of the soil temperature tank, the other is suspended below the baffle (L, fig. 6) in the stream of air flowing out of the air chamber. The temperature measured by each bulb is recorded on the seven-day chart of the recording instrument.

The control point for each bulb is set at the desired temperature, which may be the same or different, for the soil temperature tank and the air chamber of the same unit. A three-point mercury switch within the instrument is so wired that a neutral contact is made whenever the temperature is that for which the control is set. When the temperature drops below the control point, a contact is made by the three-point mercury switch which, through a relay, completes the circuit to one of the electric heaters. If the temperature rises above that for which the instrument is set, the three-point mercury switch makes a contact which opens a solenoid valve allowing the refrigerant to expand in one of the cooling coils. The differential in temperature required to throw the 3-point mercury switch to the high or low temperature side of the neutral point is adjustable.

Performance

Extensive tests to determine the performance of this equipment under the extremes of outside temperatures to which it has been exposed have not been made. The equipment, however, has been operated extensively for the purpose for which it was designated: the growing of plants at controlled temperatures. Thus from January 11 to March 10, 1937, the growth chambers were operated at air and tank temperatures of 40°, 50°, and 60° F. Outside air temperatures ranged from a low of $-6°$ to a high of 68° F. during this period. During mild, clear days, the air temperature in the 40° compartment would rise to a maximum of 50° F. at mid-day. Except during this brief period, air temperature did not vary from the setting of 40° by more than $\pm 2°$ F., or from settings of 50° and 60° by more than $\pm 2.5°$ F. At no time did the temperature of the soil temperature tanks vary from the set temperature by more than 1° F. in any of the compartments.

From March 18 to May 13, 1937, the growth chambers were operated at air and tank temperatures of 60°, 70°, and 80° F. During this period, outside temperatures ranged from 23° to 86° F. No difficulty was experienced in maintaining the temperatures for which the controls were set within a maximum range of $\pm 2.5°$ in the case of air temperatures and 1° in the case of tank temperatures.

From May 31 to July 26, 1937, the growth chambers were maintained at air and tank temperatures of 80°, 90°, and 100° F. During this period outside air temperatures ranged from 58° to 96° F. Again air temperatures within the compartments were held within a range of $\pm 2.5°$ from the set temperature, and tank temperatures within a range of $\pm 1°$ F.

The temperature ranges given above represent extreme variations which did not occur during the greater part of the time in which the growth chambers were in operation. Furthermore, except in the case of air temperature in the 40° compartment on mild clear days, the variations above and below the set temperature were equal.

From July 28 to September 20, 1936, all three soil temperature tanks were maintained at 70° F. while air temperatures were held at 70°, 85°, and 100° respectively. Variations from set temperatures during this period were no greater than previously described. Outside air temperatures ranged from a minimum of 41° F. to a maximum of 98° F. during this period.

From September 24 to November 19, 1937, the tanks were again maintained at 70° F. in all three growth chambers, while air temperature was also maintained at 70° in one compartment. In another growth chamber an air temperature of 80° was maintained during the day and one of 60° during the night. In the third growth chamber a 90° day and a 50° night air temperature were maintained. Approximately one-half hour was required to change the air temperature from the day to night or night to day setting and with the exception of this brief period, air and tank temperatures were held constant within the ranges previously given. The diurnal changes in the setting of the controls had to be made by hand, but this was a simple operation. Outside air temperatures ranged from a minimum of 18° to a maximum of 94° F. during this period.

From November 21, 1937, to January 15, 1938, the growth chambers were again operated at air and tank temperatures of 60°, 70°, and 80° F. with no greater variations from control settings than reported for previous periods. The range of outside air temperatures during this period was from 5° to 60° F.

In order to test the cooling capacity of the soil temperature tanks, one was lowered to a temperature of −10° F. and held at this level from 4 to 5 P.M. The average outside temperature on this date was 46° F.

One growth chamber was maintained at an air temperature of 60° F. from 1 to 3 P.M., May 20, 1937, a clear afternoon, with an outside wet bulb temperature of 75° and a dry bulb temperature of 88° F. No water was run over the roof of the growth chamber during this test period.

Since at no time has it been necessary to use more than one-half of the heating capacity of the air conditioning unit (two heaters controlled by separate switches are provided in each), much higher temperatures than have been applied so far can undoubtedly be attained.

During the first year of operation, mechanical failures of the equipment have been infrequent and of a minor nature. Because of the use made of standard air-conditioning and refrigeration equipment, repair parts are

readily obtained, and necessary repairs and adjustments can be made by the local refrigerator service department.

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GLYCINE IN THE NUTRITION OF EXCISED TOMATO ROOTS

PHILIP R. WHITE

(WITH TEN FIGURES)

Five years ago it was unequivocally demonstrated for the first time that an isolated plant organ, the root, could be grown in culture for potentially unlimited periods of time (**8**). An environmental complex capable of supporting normal growth for such periods was outlined which included only one factor recognized as being of unknown constitution. That factor was an extract of dried brewers' yeast representing 1/10,000 of the total mass of the nutrient. Since this one unknown made interpretations of any experiments utilizing the complex of uncertain validity, much effort has been expended since then aiming at the elimination of this factor. Analysis showed the effective material to be soluble in 85 per cent. alcohol, insoluble in ether, and separable by extraction with 100 per cent. alcohol into two fractions both of which were essential for normal growth (**10**). The fraction insoluble in 100 per cent. alcohol was shown to be replaceable by a mixture of 9 amino acids (**11**). This demonstration was obtained before the nature of the 100 per cent. alcohol soluble material, now known to be thiamin or its precursors, was known (**1, 4, 12**). A nutrient containing thiamin and amino acids was still inferior to the original yeast extract medium. It was shown to be improved by addition of a complex mixture of accessory salts. Analysis of the accessory salt mixture showed most of the ingredients to be either inert or detrimental, and by elimination of all but four (iodine, manganese, zinc, and boron), the nutrient was improved to the point where growth equal to that in a yeast extract medium could be obtained (**13**). A completely known nutrient for the maintenance of normal growth of excised tomato roots was thus made available.

The nutrient so developed was, however, rather complicated, consisting of C. P. grade sucrose, 9 amino acids, thiamin, 10 salts of C. P. grade, and redistilled water. The last step in its development had involved an analysis of the earlier accessory salt mixture and had resulted in considerable simplification. It was suggested at that time (**13**) that a similar analysis of the amino acid mixture was desirable. This was particularly so since the amino acid mixture had been developed at a time when the nutrient still included an unknown factor, the material soluble in 100 per cent. alcohol, now known to be thiamin. This paper presents the results of an investigation of the amino acid mixture previously used, in the presence of adequate amounts of thiamin and of a satisfactory supply of accessory salts. The materials and methods were those already outlined elsewhere.

Experimentation

An attempt was first made to determine if any single acid might be dispensed with. Doubt had existed from the first as to the really essential character of proline, valine, and serine. An experimental series was set up in which each of the 9 amino acids was omitted in turn from an otherwise complete nutrient. The result, shown in figure 1, was quite unexpected. Omis-

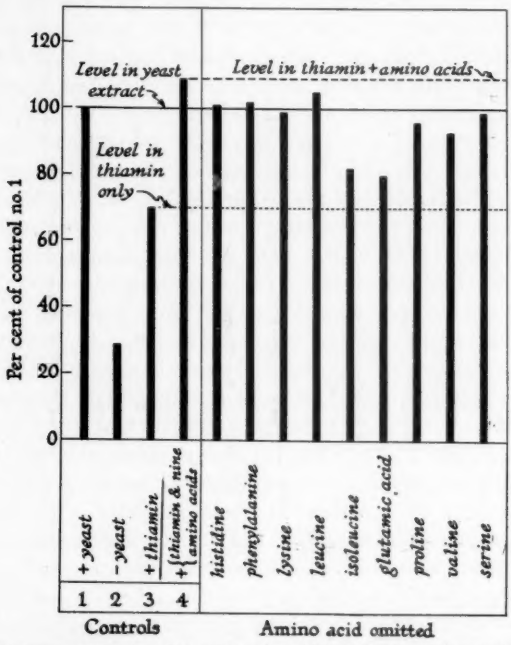

FIG. 1. Histogram showing the effect of omitting single amino acids from an otherwise complete nutrient containing standard salts, accessory salts, sugar, thiamin, 8 of the earlier 9 amino acids, and redistilled water. Only in the cases of isoleucine and glutamic acid is the effect sufficient to be of possible significance.

sion of histidine, phenylalanine, lysine, leucine, proline, valine, or serine in no case resultled in significant diminution in growth rate. Each was apparently unessential when the other 8 acids were all present. Growth in the absence of isoleucine was about 17 per cent. less than in its presence, and in the absence of glutamic acid about 20 per cent. less. But differences of less than 30 per cent. have in past work not been considered of certain significance. Even isoleucine and glutamic acid were thus of doubtful importance.

Moreover, omission of histidine and phenylalanine, histidine and lysine, histidine and leucine, histidine and isoleucine, and of lysine and leucine

likewise gave results which in no case differed significantly from the controls. It is particularly noteworthy that, although omission of isoleucine alone had

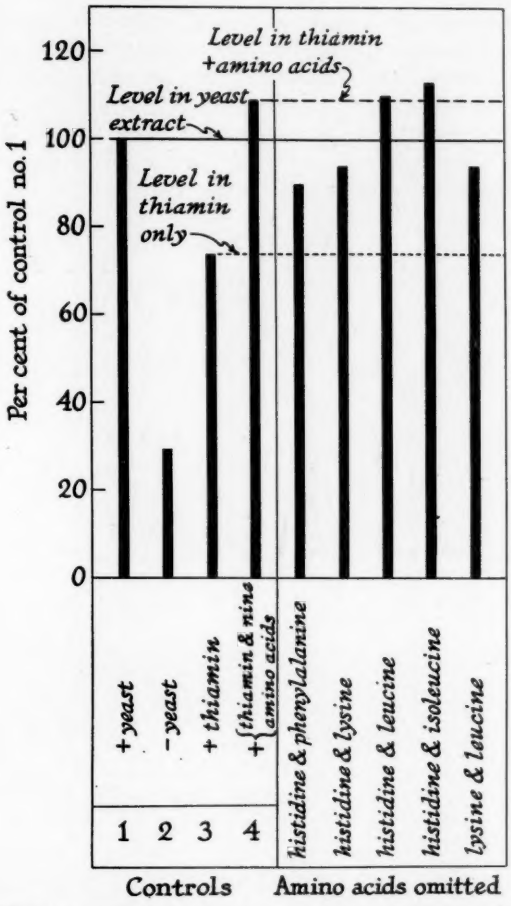

FIG. 2. Effect of omitting pairs of amino acids from an otherwise complete nutrient. In no case is the effect sufficient to be of significance.

earlier given a possibly significant decrease (17 per cent.), when both isoleucine and histidine were omitted the results were 13 per cent. better than the control. The earlier decrease was attributable to random variation due to unknown causes and was not significant. No one of these amino acids and none of the pairs tested was indispensable. Yet growth in the absence of all

9 amino acids was regularly between 30 per cent. and 60 per cent. less than in their presence. "Amino acid" seemed to be essential for normal growth but not any particular amino acid.

Since no single amino acid of the original group could be shown to be indispensable, the suspicion arose that the amino acid requirement of these roots must be far simpler than the earlier work had indicated. It may be recalled that one chart used two years ago (**11**, p. 794, fig. 1) showed that glycine alone would support growth almost equal to the control, for one passage. But when tested over a second passage, in the absence of thiamin, the growth rates dropped to practically nil (fig. 3). This, together with the

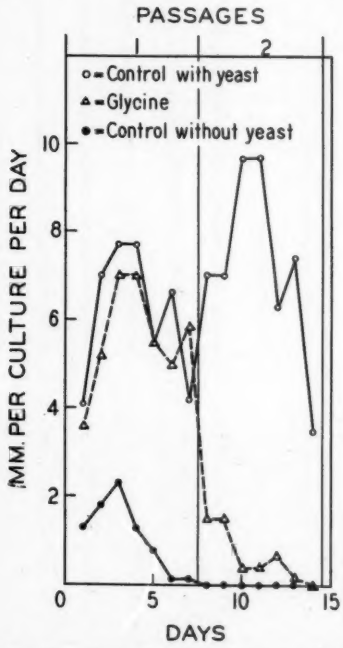

FIG. 3. Graph showing the effect of adding 5 p.p.m. of glycine to a basic nutrient containing standard salts, accessory salts, and sugar, recorded over two passages. While growth in the first passage was almost equal to that in a yeast extract nutrient, in the second passage the growth rate dropped almost to zero.

fact that addition of glycine to the 9-amino-acid mixture under consideration did not improve it, led at that time to the abandonment of the study of glycine. Now it appears that in these experiments the limiting factor was not glycine, but thiamin. It seemed desirable, therefore, to re-investigate the effects of glycine, this time in the presence of thiamin.

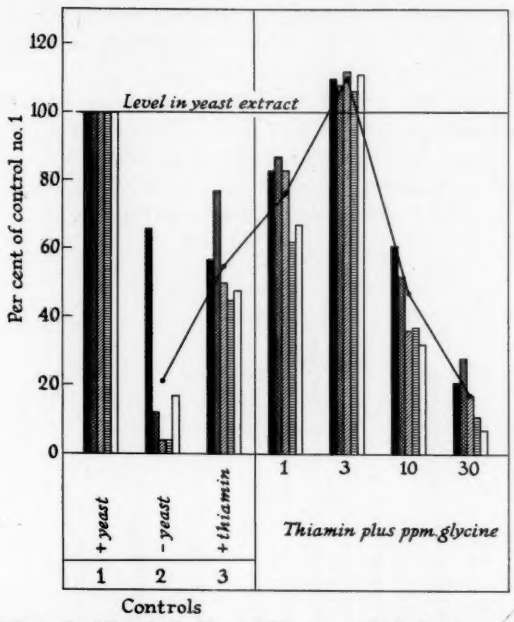

FIG. 4. Effect of adding 1, 3, 10, and 30 p.p.m. of glycine to a nutrient like that used in figure 3, but with the addition of .0.1 p.p.m. of thiamin. With 3 p.p.m. glycine, the increment exceeded that in a yeast extract nutrient in every one of the 5 passages.

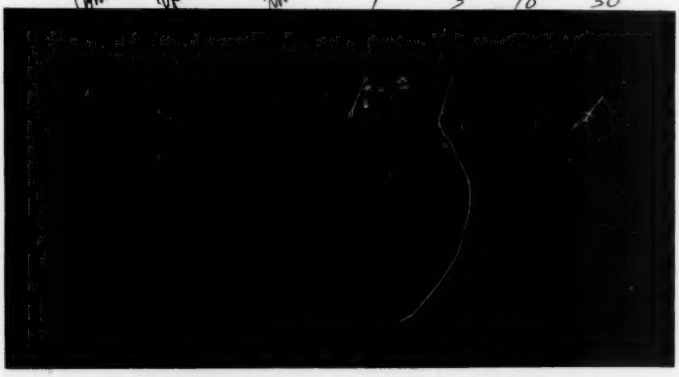

FIG. 5. Photograph of roots grown in nutrients containing in addition to H_2O, standard and accessory salts, and sugar, the following supplementary materials, reading from left to right: none (negative control); yeast extract (positive control); thiamin only; and thiamin plus 1, 3, 10, and 30 p.p.m. glycine. These roots were from passage 5 (the open columns) of figure 4. Note that roots grown in a nutrient supplemented by thiamin only were thin and obviously in poor condition. (Photograph by J. A. CARLILE.)

A concentration series in which yeast extract was replaced by accessory salts, 0.1 p.p.m. thiamin, and 1, 3, 10, or 30 p.p.m. glycine gave the results shown in figures 4 and 5. While 10 p.p.m. and 30 p.p.m. glycine were evidently injurious and 1 p.p.m. was inadequate to support normal growth, the root growth in a solution containing 3 p.p.m. glycine was superior to that in the control nutrient containing yeast extract during every one of five consecutive passages. A second concentration series fixed the optimum concentration at between 2 and 4 parts per million (fig. 6). This laboratory's

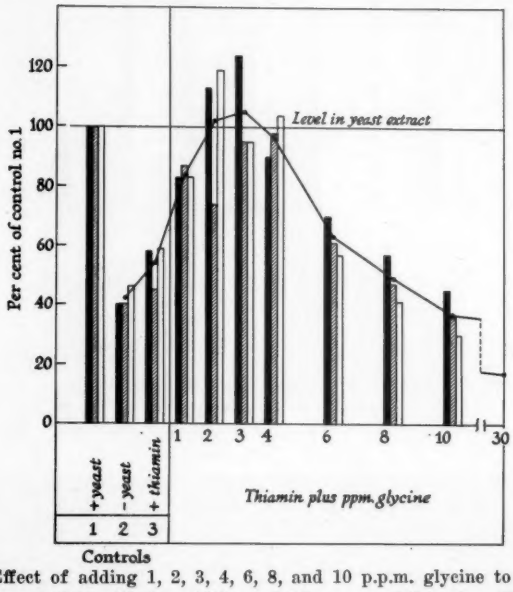

FIG. 6. Effect of adding 1, 2, 3, 4, 6, 8, and 10 p.p.m. glycine to a nutrient like that used in figure 3, but with the addition of 0.1 p.p.m. of thiamin. The optimum lies between 2 and 4 p.p.m. glycine (*cf.* fig. 4).

6-year-old stock of tomato roots was subsequently transferred to a nutrient in which the organic constituents of yeast extract were replaced by 0.1 p.p.m. thiamin plus 3 p.p.m. glycine, and grown for 11 weeks. The average growth rate of these cultures in this 11-week period was excellent and was equal to that in any previous single 11-week period except the corresponding period a year before (June–July–August, 1933 = 2.9, 1934 = 7.2, 1935 = 8.0, 1936 = 8.5, 1937 = 10.0, 1938 = 9.7 mm. per culture per day).

It is clear then, that the amino acid requirements of excised tomato roots can be supplied by the single amino acid, glycine (α-amino-acetic acid) at a concentration of 3 p.p.m. or 4×10^{-5} M. The next more complex amino acid, α-amino-propionic acid (alanine), which in the β form has been reported to

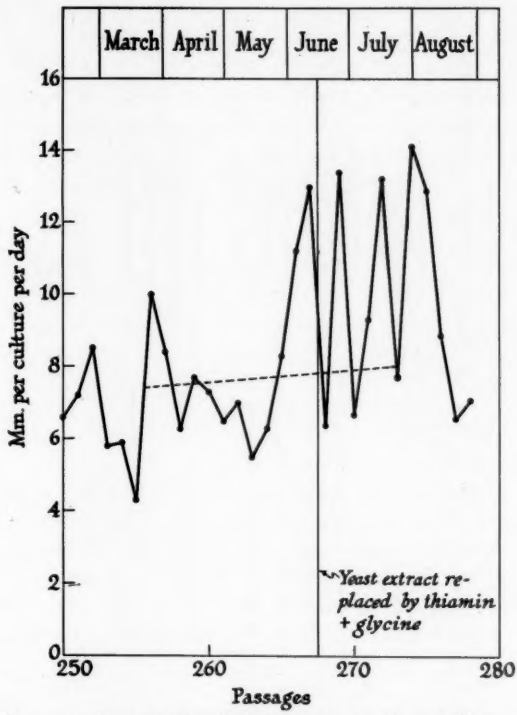

FIG. 7. Mean growth rates of excised tomato roots through 18 passages in a yeast extract nutrient followed by 11 passages in a glycine-thiamin nutrient. The dotted line indicates approximately the seasonal increase to be expected in a yeast nutrient over this period of the year (7).

be a growth requirement for some bacteria, proved extremely toxic at equivalent concentrations. Isoleucine (β-methyl-α-amino-valeric acid) was somewhat less toxic. Glutamic acid (α-amino-glutaric acid), aspartic acid (α-amino-succinic acid), and lysine (α-ε-diamino-caproic acid) showed neither a stimulating nor a depressing effect at similar concentrations. This behavior of aspartic acid is of particular interest in view of the reported rôle of its acid amide, asparagine, as the usual amino nitrogen transport substance in plant nutrition. This evidence makes such a rôle seem extremely doubtful in the case of the tomato. Although not all of the amino acids of the original list of 9 have been tested singly, it appears that the more complex forms may not be satisfactorily utilized by tomato roots for transformation into dissimilar amino acids, and that only the most simple form (α-amino-acetic acid) is available for this purpose.

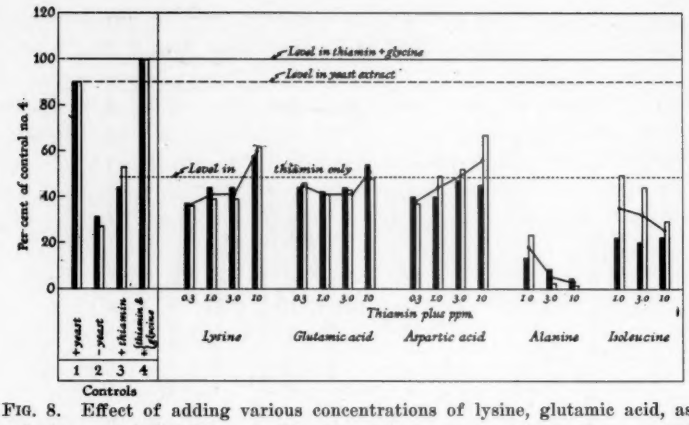

FIG. 8. Effect of adding various concentrations of lysine, glutamic acid, aspartic acid, alanine, and isoleucine to a thiamin nutrient. In no case is there a significant increase.

Amino-acetic acid has two important groups: the amino group and the carboxyl group. These experiments have shown that the amino group is not effective if attached to the propionic, valeric, caproic, succinic, or glutaric radicals. To determine if the carboxyl group would be equally effective if attached to other cations, acetic acid, citric acid, and malic acid were tested at molar concentrations equivalent to those used with glycine. The former

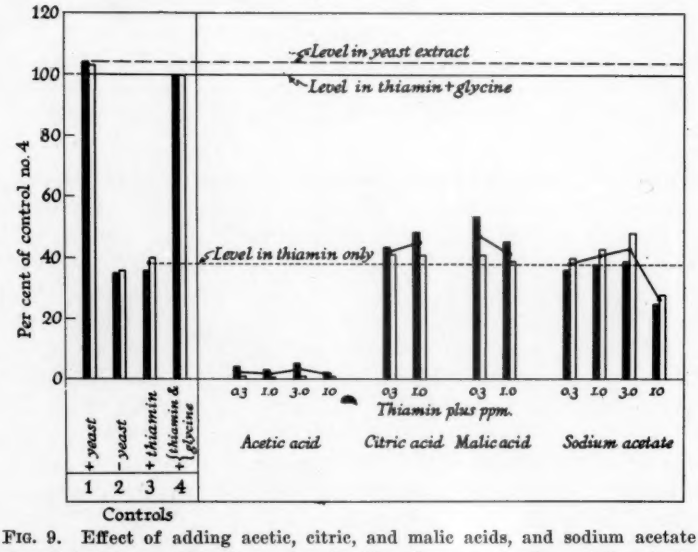

FIG. 9. Effect of adding acetic, citric, and malic acids, and sodium acetate to a thiamin nutrient in concentrations approximating those previously used for glycine.

was markedly toxic, while the latter two were without significant effect (fig. 9). The acetate portion was likewise shown to be without effect when supplied as sodium acetate. The effectiveness of glycine is, then, not owing merely to its supplying amino nitrogen, α-amino groups, carboxyl groups, or acetate. It functions neither as an organic acid nor merely as an amino acid. Glycine appears to be a growth-promoting substance *per se* rather than because of any of its components.

Discussion

Glycine alone is, then, capable of supplying all the amino-nitrogen required for growth of excised tomato roots other than that which they may be able to synthesize from inorganic nitrate. The validity of this conclusion seems unimpeachable. Yet glycine represents, according to MEISENHEIMER (**3**), only 0.5 per cent. of the entire amino acid content of yeast, and the amount of glycine present in the optimal concentration of yeast extract, calculated on this basis, is only 0.03 mg. per liter. This is 1/100 of the glycine concentration optimal for growth and would be quite inadequate in itself to support the increments observed in a yeast extract medium. It is clear that yeast extract can not owe any considerable part of its effectiveness to its glycine content, but to the particular balance of other substances present. Glycine is entirely wanting in zein, hordein, gliadin, vicillin, and some other storage proteins (OSBORNE and associates). The demonstration of its capacity to supply all of the amino acid required by excised tomato roots can not, therefore, be interpreted as in any way indicative of the nature of the substance or substances normally supplied by the green tissues of the intact tomato plant to its roots. We are apparently dealing here merely with a workable substitution.

However remote this result may be from representing the conditions in the intact plant, it is, nevertheless, a step forward in our efforts to establish a completely known nutrient for the cultivation of isolated roots since it permits at one stroke the elimination of 8 organic ingredients which might have presented difficulties when it came to removing the last traces of impurities from the nutrient. The organic constituents of the nutrient are reduced to three: sucrose, thiamin, and glycine. In our present state of knowledge, these or their equivalents can not be omitted, although ROBBINS and BARTLEY (**5**) and BONNER (**2**) have shown that in some cases the thiamin can be replaced by the simpler thiazole or pyrimidine, or by a mixture of both. ROBBINS and SCHMIDT (**6, 7**) have presented evidence that vitamin B_6 may benefit growth of excised tomato roots in a solution containing nutrient salts, sucrose, and thiamin. The optimal concentration of vitamin B_6 appears to be about 1 mg. per liter (50 γ per flask containing 50 ml. of nutrient). The growth increase produced by this addition is reported to be about 100 per cent.

The present paper shows that about the same increase is obtained when 3 mg. of glycine are added in place of 1 mg. vitamin B_6. While differences in method preclude any direct comparison between the results of ROBBINS and SCHMIDT and those reported here, it is interesting to note that glycine and vitamin B_6 appear to have, weight for weight, growth-promoting properties of about the same magnitude. Experiments to test the effects of these two substances in the presence of one another are now in progress. Since both thiamin and glycine are required in extremely small amounts and are both synthetic products, only the sucrose remains as a possible source of unknowns. Repurification of these crystalline ingredients represents, it may be hoped, the last step in the establishment of a completely known nutrient for the cultivation of excised tomato roots.

Caution must be employed, however, in extending these conclusions to species other than tomato. This laboratory maintains root cultures of a number of other plants. Naturally, the glycine-thiamin nutrient was tried on them. Most species do well. But, as figure 10 shows, such a nutrient

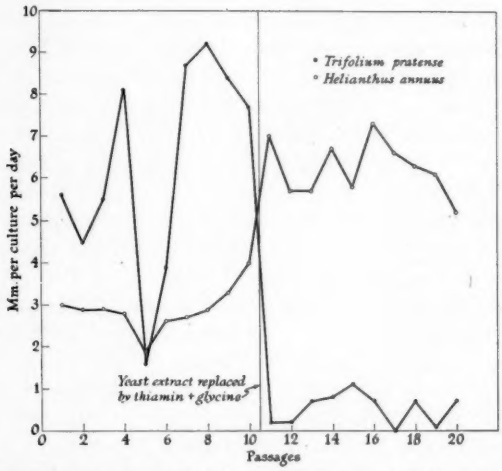

FIG. 10. Behavior of roots of *Trifolium pratense* L. and *Helianthus annuus* L. in nutrients in which yeast extract was replaced by thiamin, glycine, and accessory salts.

does not support growth of clover, while sunflower roots do much better in it than in a yeast nutrient. Roots of legumes and of composites behave quite differently. These differences present in themselves vast fields for research.

Summary

Analysis of the 9 amino-acid mixture earlier developed as a constituent of

the nutrient for cultivation of excised tomato roots showed that no single amino acid and none of a number of pairs of acids was indispensable. Glycine alone proved capable of replacing the entire group. Neither the α-amino group, the carboxyl group, nor the acetate radical was effective when supplied in any of several related compounds. Since glycine is not present in many storage proteins and is present only in small amount in yeast, no conclusions as to the form in which amino acid may be supplied to roots in the intact plant can be drawn from this result. A completely known and relatively simple nutrient is now available for the cultivation of excised tomato roots. This nutrient as now constituted contains 20 gm. sucrose, 100 mg. calcium nitrate, 35 mg. magnesium sulphate, 80 mg. potassium nitrate, 65 mg. potassium chloride, 12.5 mg. potassium acid phosphate, 0.75 mg. potassium iodide, 2.5 mg. ferric sulphate, 4.4 mg. manganous sulphate, 1.5 mg. zinc sulphate, 1.6 mg. boric acid, 3 mg. glycine and 0.5 mg. thiamin per liter. This nutrient is capable of supporting continued and probably unlimited growth of tomato roots. Further study may perhaps still further simplify this solution.

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EFFECTS OF CATIONS AND ANIONS ON PROTOPLASMIC ELASTICITY[1]

HENRY T. NORTHEN AND REBECCA T. NORTHEN

Introduction

It is generally believed that one of the chief rôles of cations is to maintain the protoplasmic colloids in the proper state of dispersion and aggregation. Many authors have compared protoplasm to non-living colloidal systems and assume that the electric charge of the colloidal particles is of first significance in preventing the precipitation of the particles.

If the charge on the protoplasmic colloids is of prime importance in maintaining the stability of the system, the valence of introduced cations should largely determine the effects of such ions in dispersing or aggregating the colloids. A greater dispersion of the colloids should result in a decrease in protoplasmic consistency and an aggregation should result in an increase. As yet, there is no general agreement as to the effects of cations on protoplasmic consistency. SEIFRIZ (**7**) states that "the effect of sodium and of calcium on protoplasm is a controversial subject, though the consensus of opinion appears to indicate that, in general, sodium disperses and calcium aggregates." On the other hand, HEILBRUNN (**1**) maintains that sodium and potassium increase the viscosity of the main mass of protoplasm whereas calcium and magnesium decrease the viscosity. In Amoeba, however, the cortical protoplasm does not respond to cations as does the endoplasm (**1**). In the cortical protoplasm HEILBRUNN reports that sodium, potassium, and magnesium cause liquefaction whereas calcium causes a pronounced stiffening.

Previous investigations carried out in this laboratory indicate that the protoplasm in which the chloroplasts of Spirogyra are embedded is not a simple colloidal system such as the physical chemist studies. For example, NORTHEN (**2**) has noted that when protoplasm in cells of Zygnema and Spirogyra is cooled the consistency reaches a maximum as rapidly as temperature equilibrium is attained, whereas in non-living hydrophilic colloidal systems (*e.g.*, gelatin solution) the consistency increases during a long period after temperature equilibrium has been reached. One assumption of NORTHEN, however, should be corrected. At the time, NORTHEN stated that it was probable that the displacement of the chloroplasts by centrifugal accelerations was limited by the ease with which the chloroplasts could bend rather than by the resistance of the cytoplasm. Observations on the appearance of cells following centrifugation clearly indicate that the elasticity of the cytoplasm is the limiting factor rather than the ease with which the chloro-

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 171.

plasts are bent. Following centrifugation the main mass of the chloroplasts are aggregated at the centrifugal end of the cell but the ends of the chloroplasts extend back (have "unbent") from the aggregated mass (see illustrations in HEILBRUNN **1**, page 76, and in NORTHEN **2**, page 195). The extended portions indicate that the cytoplasm is impeding their motion, so much so that they are straightened out.

NORTHEN (**3**) has found that the protoplasm in which the chloroplasts of Spirogyra are embedded is an elastic fluid and has deduced (**6**) that protoplasm has a net-like ultramicroscopic structure. Supposedly the network results from the combination of proteins with lipoids and carbohydrates. It is probable that many inorganic ions are tied up to the network or occupy key positions in the network such as magnesium does in the chlorophyll molecule. The network is a labile structure rather than a stable one. The structure is readily altered by injury (**4**), dilute solutions of anesthetics (**5**), and by temperature (**6**). We doubt if any known non-living system can respond to the above stimulants as does protoplasm. The responses of protoplasm are unique and hence it is reasonable to believe that protoplasmic responses to cations and anions will also be unique.

According to the preceding concept the protoplasmic system is semi-stable because molecules have definite places in the network and because they are not in suspension. For example, protein molecules in the network can aggregate only when they are liberated from the other constituents of the network (go into colloidal suspension), and of course then only when other requisites of aggregation are favorable. In a structure such as postulated, the chemical properties of an ion will be of more importance in altering the structure than will be the valence of the ion. The data presented will show that the valence of introduced cations or anions has little effect on the structure of the protoplasm.

A discussion of the effects of cations on protoplasm should include mention of ion antagonism. It is common knowledge that the toxic effects of the sodium ion can be counteracted by the calcium ion. According to SEIFRIZ (**7**) the univalent sodium ion liquefies protoplasm and the divalent calcium ion aggregates, but when the two are mixed together the protoplasm remains normal. Assuming that Na and Ca act as SEIFRIZ states, that type of antagonism can be explained on the basis of charge carried by the ions. The antagonism of the divalent magnesium ion by the divalent calcium ion or the divalent strontium ion (**8**) cannot be explained, however, on such a basis. The data presented will show that the magnesium ion tightens the protoplasmic structure (increases elasticity) and that both the calcium and strontium ions loosen the network (decrease elasticity). The calcium ion and to a lesser degree the strontium inhibit the effect of the magnesium ion. One cannot, however, always predict from a study of single cations what the effect

will be when two ions are allowed to act at the same time. For example, it will be demonstrated that both magnesium and cadmium when acting singly cause a decided increase in elasticity but that when cadmium and magnesium act together they produce a decrease in elasticity.

Method

The method is based on the equation: $V = k(c - c_o)$, which approximately governs the rate with which the chloroplasts in cells of Spirogyra move in response to different centrifugal accelerations (**3**). In the above equation V is the velocity of chloroplastic movement, k a constant, c the centrifugal acceleration used, and c_o is the initial starting centrifugal acceleration at which, or below which, the chloroplasts will not move regardless of how long the acceleration is allowed to act. To understand the meaning of the above equation, imagine a test-tube filled with an elastic fluid, a fluid in which long intermeshed molecules form a network. If a ball is now introduced, it will not sink because the net holds it up. Next centrifuge the tube. With low accelerations the ball will not move, regardless of how long the tube is centrifuged, because the low centrifugal acceleration acting on the ball does not create a sufficient force to shear apart the interlaced molecules. If the acceleration is increased so as to produce the necessary shearing force, the ball will move. The maximum acceleration which is not sufficient to shear apart the interlaced molecules has been designated as c_o. With accelerations greater than c_o the ball will move. Likewise, in cells of Spirogyra the network must be sheared apart before the chloroplasts can move through the cytoplasm.

Variability characterizes organisms. In a population of Spirogyra c_o is not the same for all of the filaments. Hence when a number of filaments are centrifuged with an acceleration of $680 \times$ gravity, the chloroplasts will be displaced in cells of filaments whose value of c_o is less than $680 \times$ gravity and will not be displaced in cells in which the value of c_o is equal to or greater than $680 \times$ gravity because $V = k(680 - 680) = 0$ or $V = k(680 - > 680) = < 0$. Any treatment which loosens the structural network will decrease the value of c_o, and accordingly when treated filaments are centrifuged with an acceleration of $680 \times$ gravity the chloroplasts will be displaced in more cells than in untreated ones. On the other hand any treatment which tightens the structure will increase the value of c_o and concomitantly decrease the percentage of cells in which the chloroplasts are displaced (moved to centrifugal end). A decrease in c_o has been termed a decrease in elasticity, whereas an increase in c_o has been interpreted as an increase in elasticity.

Filaments of Spirogyra were immersed for one- and two-hour intervals in solutions of various cations and anions, and in some experiments in solutions of mixed cations. To minimize osmotic effects approximately isosmotic solutions have been used. If the molecules dissociated into two ions 0.07 M

solutions were used; if into three ions 0.05 M solutions were used; and if into four ions 0.035 M solutions were used. After the filaments had been in the solutions for the desired time they were placed between strips of cotton soaked in the appropriate solution and centrifuged with an acceleration of 680× gravity for thirty seconds. Following centrifugation the percentages of filaments in the cells of which the chloroplasts had been displaced were

TABLE I

EFFECTS OF UNIVALENT AND DIVALENT CATIONS ON PROTOPLASMIC ELASTICITY

GROUP I	GROUP II
Hydrogen*	
D Lithium 75 per cent.—1 hour 86 per cent.—2 hours	I Beryllium 6 per cent.—1 hour 1 per cent.—2 hours
I Sodium 32 per cent.—1 hour 20 per cent.—2 hours	I Magnesium 8 per cent.—1 hour 6 per cent.—2 hours
D–I Potassium 85 per cent.—1 hour 16 per cent.—2 hours	D Calcium 93 per cent.—1 hour 61 per cent.—2 hours
I Copper 0 per cent.—1 hour 0 per cent.—2 hours	I Zinc 0 per cent.—1 hour 0 per cent.—2 hours
Rubidium*	D Strontium 80 per cent.—1 hour 71 per cent.—2 hours
I Silver 0 per cent.—1 hour 0 per cent.—2 hours	I Cadmium 0 per cent.—1 hour 0 per cent.—2 hours
O–D Caesium 46 per cent.—1 hour 44 per cent.—2 hours	D Barium 100 per cent.—1 hour 100 per cent.—2 hours
Gold*	I Mercury 0 per cent.—1 hour 0 per cent.—2 hours

Distilled Water	Tap Water	O = little or no effect
52 per cent.—1 hour	73 per cent.—1 hour	I = elasticity increased
30 per cent.—2 hours	73 per cent.—2 hours	D = elasticity decreased

* Ion not used.

determined. For each experimental group between 400 and 500 filaments were examined.

Data and discussion

The effects of univalent and divalent cations on the elasticity of protoplasm in Spirogyra are recorded in table I. The sulphate salt of beryllium and the nitrate salt of silver were used. In all other instances the chlorides were used. In the table the elements are arranged as in the periodic table. Below each element the percentages of filaments in the cells of which the chloroplasts were displaced by centrifugation after one hour's immersion and two hours' immersion are recorded. The letter O above an ion indicates that when compared to distilled water (the solvent) that ion had little effect. The letter D indicates that the ion decreased protoplasmic elasticity and the letter I indicates that the ion increased the elasticity. It should be remembered that an increase in elasticity indicates a tightening of the network and a decrease in elasticity indicates a loosening of the network.

It will be noted, table I, that the valence of the ion has little effect in determining whether the elasticity will be decreased or increased. For example, the monovalent lithium ion decreases elasticity whereas the monovalent sodium ion increases it.

In group II it will be noted that the ions of chemically similar elements act approximately alike. Chemically beryllium, magnesium, zinc, cadmium, and mercury are related and their ions cause an increase in the elasticity of protoplasm. Chemically calcium, strontium, and barium are closely related and their ions act alike in decreasing protoplasmic elasticity. Hence it appears that changes in protoplasmic structure are correlated with the chemical nature of the divalent cation, and accordingly the effects of the divalent cations on the stability of the protoplasm seems to be determined by chemical properties rather than by such properties as valence.

Although in group I lithium, sodium, potassium, and caesium are related, they do not affect protoplasm in a like manner. After two hours' immersion the very closely related sodium and potassium ions cause a slight decrease in elasticity. (Differences of less than 8 per cent. are not considered significant because of the variability of the samples.) The caesium ion causes a slight decrease in elasticity whereas the lithium ion causes a pronounced decrease. We do not know why caesium should act differently from sodium and potassium. Typically the first member of a family has many properties which are not shared by subsequent members. Perhaps some of these unique properties explain the liquefying effect of the lithium ion. Chemically copper is related to silver and has much in common with mercury and their ions have a similar action on protoplasmic structure.

Most cations affected protoplasmic structure in one way or another. This

indicates that the cations penetrated, although it is not known how much of each actually did enter the cells. The data suggest, however, that the effects of the cations is chemical, which implies that the cations react in some unknown way with the network constituents. The fact that the ions probably react with network constituents should be considered in studies of permeability and accumulation of ions. Certainly the effects of the cations are not the result of the ions just passing through the cytoplasm. Hence it is likely that some types of cation accumulations are the result of reactions between the cations and protoplasmic constituents.

If one ion decreases protoplasmic elasticity and another increases elasticity, it might be assumed that when the two ions act together the protoplasmic structure will remain approximately normal. That such is not always true, however, is shown by the data recorded in table II, in which experiment filaments of Spirogyra were immersed in a solution containing magnesium chloride plus some salt of another divalent cation.

TABLE II

EFFECTS OF CATION COMBINATIONS ON PROTOPLASMIC ELASTICITY

SOLUTION	PERCENTAGES OF FILAMENTS IN THE CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED AFTER IMMERSIONS OF	
	ONE HOUR	TWO HOURS
	%	%
Tap water	49	49
Distilled water	38	25
0.05 M $MgCl_2$	4	2
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.07 M $BeSO_4$	13	3
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.05 M $CaCl_2$	31	28
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.05 M $ZnCl_2$	0	0
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.05 M $SrCl_2$	10	4
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.05 M $CdCl_2$	37	57
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.05 M $BaCl_2$	90	80
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.07 M $BeSO_4$	12	
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.05 M $CaCl_2$	27	
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.05 M $ZnCl_2$	0	
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.05 M $SrCl_2$	17	
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.05 M $CdCl_2$	36	
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.05 M $BaCl_2$	77	

Table II demonstrates that the magnesium ion can be decidedly antagonized by the barium, cadmium, and calcium ions. Such results are of especial interest because both the cadmium and magnesium ions by themselves cause an increase in elasticity. In proportions of 9 to 1 the strontium ion exhibits a slight antagonistic effect after one hour's immersion.

The data in table I indicate that magnesium, through chemical changes in

the protoplasmic network, causes an increase in the elasticity of the protoplasm, and the data in table II indicate that the calcium ion can antagonize this effect when the two ions are acting at the same time. The data recorded below demonstrate, however, that once the protoplasmic structure has been decidedly altered by the magnesium ion it cannot be brought back to normal by the calcium ion. In this experiment filaments were immersed for one hour in 0.05 M $MgCl_2$. Next they were transferred to 0.05 M $CaCl_2$ solution where they were allowed to remain for intervals of one and two hours before centrifugation.

Treatment	Percentage of filaments in the cells of which the chloroplasts were displaced
	%
$MgCl_2$ for one hour	8
$CaCl_2$ for one hour	52
$MgCl_2$ for one hour—$CaCl_2$ for one hour	5
$MgCl_2$ for one hour—$CaCl_2$ for two hours	2

The effects of the potassium salts of various anions on protoplasmic elasticity are recorded below. Underneath each ion are recorded the percentages of filaments in the cells of which the chloroplasts were displaced by centrifugation. The figures in parentheses are the percentages of filaments in which the chloroplasts were displaced after an immersion of one hour and the figures which are not in parentheses are the percentages after an immersion of two hours.

$CrO_4^=$	F^-	$PO_4^=$	$SO_4^=$	Br^-	I^-	Cl^-	Dist. H_2O	NO_3^-	Tap H_2O	$AsO_4^=$
(22%)	(62%)	(23%)	(33%)	(35%)	(40%)	(26%)	(38%)	(80%)		(43%)
6%	9%	16%	20%	23%	27%	28%	30%	50%	51%	57%

When compared with distilled water it will be noted that the nitrate and arsenate ions decrease protoplasmic elasticity. The closely related bromide, chloride, and iodide ions have little effect on elasticity. After two hours' immersion the sulphate, phosphate, fluoride, and chromate ions cause an increase in protoplasmic elasticity. It will be noted that the charge carried by the ions does not determine whether the elasticity will be increased or decreased.

The studies of the effects of cations and anions lend more support to an ultramicroscopic network structure for protoplasm than to a suspension of hydrophobic and hydrophilic colloids whose stability is determined in part by the charge of the suspended particles. An explanation of how the cations and anions affect protoplasmic structure cannot be given at the present time. It should be remembered, however, that there are metallo-organic compounds in cells. In the postulated network, ions may occupy key positions. The

substitution or removal of these ions by others cannot but have an effect on protoplasmic structure and biological processes (*e.g.*, the substitution of copper for magnesium alters the properties of chlorophyll).

Summary

1. Groups of Spirogyra filaments were immersed for one- and two-hour intervals in isosmotic solutions of various cations, anions, and mixed cations. Controls were maintained in distilled and tap water. They were then centrifuged in the appropriate solution with an acceleration of 680 × gravity for 30 seconds. Comparison of the percentages of filaments in the cells of which the chloroplasts were displaced showed whether the elasticity had been increased or decreased. A decrease in elasticity was interpreted as a loosening of the protoplasmic network, and an increase as a tightening.

2. The univalent cations lithium and caesium decreased protoplasmic elasticity, while sodium and potassium increased it. Of the divalent cations, chemically related calcium, strontium, and barium decreased elasticity, whereas chemically related beryllium, magnesium, zinc, cadmium, and mercury increased it.

3. The closely related anions bromide, chloride, and iodide had little effect on elasticity; the sulphate, phosphate, fluoride, and chromate ions caused an increase, and the arsenate and nitrate ions a decrease.

4. From the above data it was concluded that the effect of an ion in decreasing protoplasmic elasticity is due to its chemical activity rather than to its valence.

5. When filaments were immersed in mixtures of divalent cations, the result could not always be predicted from the behavior of the individual ion. For instance magnesium, which caused an increase in elasticity, was antagonized by barium which caused a decrease. However, magnesium was also antagonized by cadmium which individually acted like magnesium.

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CHARACTERISTICS OF THE TYROSINASE SYSTEM IN POTATOES WHICH BLACKEN AFTER BOILING[1]

A. Frank Ross, W. E. Tottingham, and Rudolph Nagy

(WITH THREE FIGURES)

The discoloration of white or Irish potatoes after cooking appears to be associated with both instability of the protein and unusual activity of the tyrosinase system (**11, 13**). Merkenschlager (**7**) attributed the abnormality jointly to accumulation of tyrosine and unusual activity of tyrosinase. This difference from normal tubers is correlated with more rapid reddening and subsequent greater darkening of frozen, ground tissue that is exposed to the air while thawing. The present paper covers an investigation of some of the enzymic factors that may contribute to this abnormality.

Experimentation

Tuber groups were selected that were uniform in appearance but different in respect to discoloration after cooking, as shown by testing longitudinal sections. These were washed without soaking, rinsed with distilled water, and dried. They were then frozen with solid carbon dioxide, ground in a meat cutter, and allowed to thaw under nitrogen. The latter precaution prevented melanin formation and was taken because of Raper and Wormall's (**10**) finding that formation of melanin at pH 6 sometimes results in the precipitation of a melanin-enzyme complex. When completely thawed, the tissue was pressed and the sap was stored under nitrogen at 0° until used. During this period starch settled out. Unless otherwise stated, borate, phosphate, and phthalate buffers were prepared as directed by Clark (**2**), with the exception that sodium salts were substituted for those of potassium in all cases.

Determination of tyrosinase activity

A modification of the method of Raper and Wormall (**10**) was used. Ten-ml. portions of press sap were placed in 500-ml. round bottom flasks containing 100 ml. of a buffered 0.05 per cent. tyrosine solution, 10 ml. of toluene and a few drops of capryl alcohol. The flasks were placed in series for aeration in a water bath maintained at 20° C. A strong current of air, saturated with water, toluene, and capryl alcohol at 20° C. was drawn through the system. At the beginning and at intervals, 5-ml. portions of the test fluid were removed, acidified with 1 ml. of 5 per cent. acetic acid, placed in a boiling water bath for 15 minutes, plugged with cotton, and

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allowed to stand twenty-four hours. They were then filtered, and the precipitate of melanin and protein washed with hot, dilute acetic acid. Thirteen ml. of a saturated sodium carbonate solution were added and the samples allowed to stand one hour. Five ml. of phenol reagent were then added, the sample was diluted to 50 ml., and tyrosine determined as directed by FOLIN and MARENZI (3) for tryptophane. The rate of loss of tyrosine was taken as a measure of tyrosinase activity.

In determining the influence of boiled juice a portion of the press sap was placed on a boiling water bath, stirred until flocculation of protein was complete, filtered, and cooled. When testing the ash, a portion of the sap was evaporated to dryness on a steam bath under a current of air, then ashed carefully. The ash was dissolved in dilute hydrochloric acid, adjusted to the desired pH with sodium hydroxide, and made up to the original volume. An amount of either boiled juice or dissolved ash equivalent to the amount of crude enzyme preparation (10 ml. of press sap) was then added to the reaction flask and an equivalent amount of distilled water to the controls.

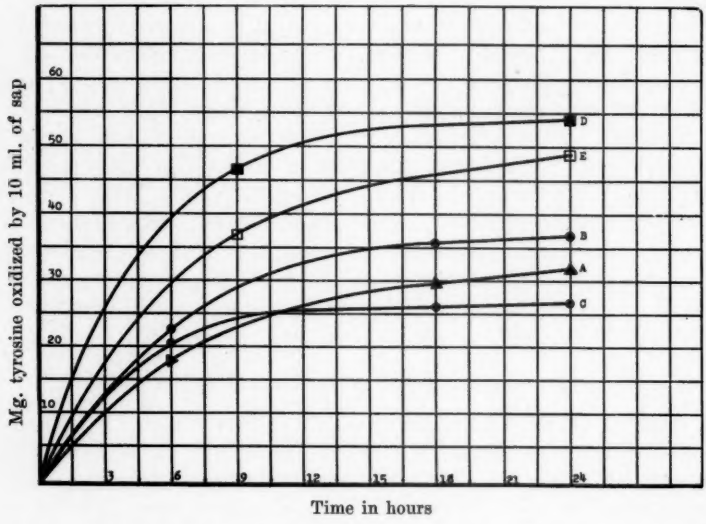

FIG. 1. Comparison of tyrosinase activity in different buffers and at different pH values.

A—unbuffered.
B—0.05 M phosphate at 6.0 pH.
C—0.05 M borate at 6.0 pH.
D—0.05 M phosphate at 8.0 pH.
E—0.05 M borate at 8.0 pH.

Effect of different buffers

Early in this investigation it was found that the buffer used had a marked influence on the activity of the enzyme. Figure 1[2] illustrates the relative activity of tyrosinase preparation in the presence of 0.05 M phosphate and 0.05 M borate at pH values of 6.0 and 8.0. The pH of the unbuffered control (5.97) did not change during the course of the reaction. At pH 6.0 phosphate accelerated the reaction continuously while borate apparently produced an initial accelerating action, which declined sharply after a lapse of several hours. In view of the results at pH 6.0 it seems reasonable to suppose that the difference in activity at pH 8.0 may be attributed both to acceleration by phosphate and to retardation by borate.

An inhibiting action was exerted by phthalate at pH 6.0 throughout the course of the reaction. Acetate and citrate buffers (0.05 M) at pH 6.0 had no effect during the first several hours, but in later stages the reaction ceased. It is believed that this was caused by removal of the enzyme by combination with the melanin, which precipitates at that pH. A mannitol-boric acid buffer prepared according to Britton (1) had no effect at pH 7.0.

The concentration of the buffer influenced the rate of enzyme action. Tyrosinase preparations were slightly less active in 0.1 M than in 0.05 M phosphate but a similar increase in borate concentration had a marked inhibitory effect. In some cases enzymatic action was arrested completely by 0.5 M borate. The color changes accompanying the oxidation of tyrosine are much more rapid in phosphate than in borate buffers. In the latter the red stage is prolonged. Unbuffered solutions adjusted to the same pH exhibit intermediate changes.

Tyrosinase activity in normal and discoloring tubers

Some doubt was cast upon the validity of our earlier determinations of tyrosinase activity (13) by the discovery that the borate buffers used were ineffective in preventing changes in pH. Addition of potato sap to borate buffer of pH 8.0, in the proportions used earlier, resulted in a final pH of 7.0 or lower. The addition of sap of abnormal tubers caused a smaller change than did that of normal tubers, but this difference was seldom over 0.2 pH.

The relative rates of oxidation of tyrosine in phosphate solutions of pH 6.95 and 7.8 were determined. This increase in pH of almost one unit produced an activity in sap from normal tubers equivalent to that in juice of potatoes which discolor. The effect of pH in borate solutions was not tested but comparison of the rates obtained in different experiments gave no indication of greater sensitivity to pH changes.

For more conclusive evidence on the relative tyrosinase activity in nor-

[2] The authors are indebted to Jerry W. Marek for preparation of the graphs.

mal and discoloring tubers, determinations in which the pH was carefully controlled were made on several lots of potatoes. The pH was adjusted by addition of sodium hydroxide and was determined in each case at the beginning and at the end of an experiment by means of a glass electrode. The results of experiments using both phosphate and borate solutions at pH 7 showed markedly greater oxidation of tyrosine by the sap of abnormal tubers. Similar results were obtained with phthalate, acetate (pH 6.0), mannitol-boric acid (pH 7.0), phosphate (pH 8.0), and unbuffered solutions. In every case the tyrosinase in press sap of discoloring tubers was more active than that of normal ones, regardless of the buffer used or of the pH at which the experiment was conducted.

These results show rather conclusively that potatoes which discolor when cooked possess an unusually high tyrosinase activity. The fact that a difference in rate of oxidation of tyrosine is always obtained, regardless of the buffer or of the pH used in testing, is further evidence that we are measuring an actual inequality either of the states of activation or of the quantities of enzyme in the two kinds of tubers. It is possible that any one buffer might react with some component of one type of potato sap, causing the formation of a complex that affects the enzyme but is non-existent in the tuber itself. If such were the case, it seems unlikely that the same effect would be produced by all the buffers used at the various pH values.

Comparative effect of buffers on the tyrosinase of normal and discoloring tubers

It was observed that the greatest differences in tyrosinase activity between normal and discoloring tubers were evident in the unbuffered solutions near pH 6.0 (the pH of the sap) and in certain concentrated buffers. The addition of phosphates, borates, or phthalates of 0.05 M concentration tended to decrease the divergence. When the phosphate concentration was increased to 0.1 M, the difference was further lessened. A similar increase in borate concentration gave the opposite effect. The borate exerted a greater inhibiting action on the tyrosinase in the sap of normal tubers than on that in discoloring ones, resulting in an accentuation of the difference between the two. A still further increase in the concentration of the borate buffer resulted in a complete inactivation of the enzyme in the juice of normal tubers, while that in the sap from discoloring tubers remained moderately active. In one experiment the latter oxidized 18.4 mg. of tyrosine in 24 hours in 0.5 M borate as compared to 33.4 mg. when in 0.1 M borate. This effect may be either partly or entirely due to a difference in enzyme quantity or state of activation, to factors in the sap that modify the action of the buffer on the enzyme, or to other factors whose effect on the enzyme is modified by the buffer. Thus experiments with purified enzyme preparations might give entirely different results.

Effect of tuber factors on tyrosinase activity

Haehn (5) noticed that boiled potato juice had an activating effect on tyrosinase. He attributed this response to the presence of certain salts and seems to have neglected the effect of additions of the ash upon pH. Raper and Wormall (10) also presented evidence for the presence of an activator in boiled sap, but found that it was not present in the ash. In view of these results, we believed it possible that the presence of some such activator in the one, or perhaps of an inhibitor in the other, might account for the difference between discoloring and normal potatoes.

An activator of tyrosinase was found to be present in boiled juice of abnormal tubers. The addition of such juice to a tyrosine-tyrosinase system caused a marked acceleration in the rate of enzyme action, while the boiled juice of normal potatoes had little or no activating effect. This effect was greatest in unbuffered solutions, slightly less marked in borate, less apparent in phthalate and mannitol-boric acid buffers, and non-existent in phosphate.

The activating effect of boiled juice is illustrated in figures 2 and 3 which summarize the results of several experiments. Maximum acceleration due

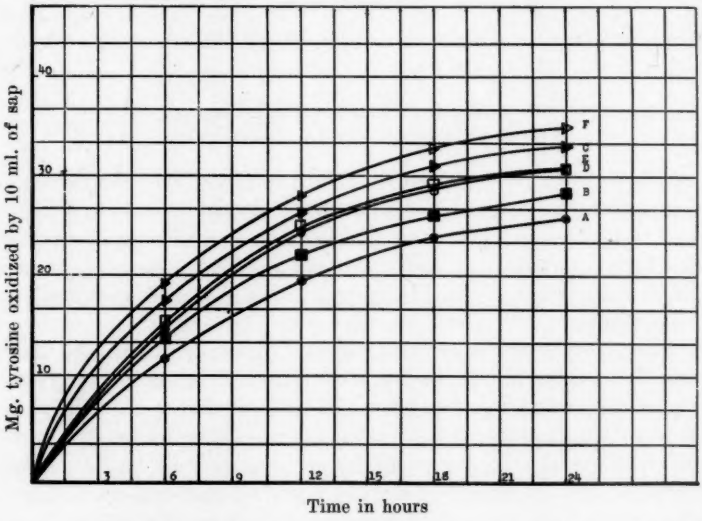

Fig. 2. Effects of boiled sap on tyrosinase activity in 0.05 M borate buffer at pH 8.0.
A—Normal sap.
B—Normal sap + boiled normal sap.
C—Normal sap + boiled discoloring sap.
D—Discoloring sap.
E—Discoloring sap + boiled normal sap.
F—Discoloring sap + boiled discoloring sap.

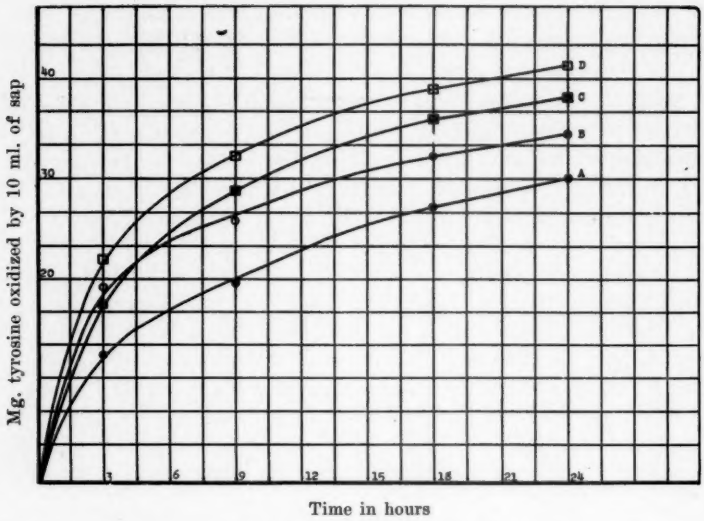

FIG. 3. Effect of boiled sap on tyrosinase in unbuffered solutions at pH 6.0.
A—Normal sap.
B—Discoloring sap.
C—Normal sap + boiled discoloring sap.
D—Discoloring sap + boiled discoloring sap.

to boiled juice was obtained when the press sap of normal potatoes was used as the source of the enzyme. It was noted that no activator could be detected in potatoes that cooked white, while those that cooked slightly grey often contained the activator in limited amounts. Some of these samples exerted a slight inhibitory action, but the effect was never of such magnitude as to be of significance.

In considering only the results of figure 2, one might conclude that this activator is the only factor responsible for the difference in the tyrosinase activity of the two types of tubers. That this probably is not the case is indicated by the fact that the activator has no effect in phosphate-buffered systems, yet the sap of abnormal tubers is always higher in tyrosinase activity than that of normal ones when tested in such buffers. There was evidence that borates also affect the activator. A greater acceleration due to the addition of boiled juice was apparent in 0.1 M than in 0.05 M borate. In 0.5 M borate the activating effect is yet more pronounced. An enzyme preparation from normal tubers that showed no tyrosinase activity in 0.5 M borate was moderately active if boiled juice from discoloring tubers was added.

The effect of the activator is less apparent at higher pH values than at lower ones. Its nature is as yet unknown. It is rather unstable, as its ability to affect the enzyme lessens upon standing at temperatures as low as 5°. It is partially destroyed by prolonged boiling. When boiled juice containing the activator was dialyzed against distilled water at 5° C. or lower, its ability to activate was lost more rapidly than when stored at the same temperature. The activator is seemingly organic in nature, for it could not be detected in the ash. It is possible, however, that an active inorganic constituent would be altered during the ashing process and thus become inactive. The fact that the compound is unstable also is an indication that it is not an inorganic salt.

The possibility that the accelerating effect of boiled juice might be caused by the slightly increased substrate concentration was considered, for the boiled juice added contained some tyrosine. In some experiments, tyrosine was added to the controls so that their final substrate concentration was equal to, or in excess of, that in the test solution. The results were essentially the same as those previously obtained. It could also be possible that the apparent acceleration was due to oxidation of compounds in the juice that are more rapidly oxidized than tyrosine itself. In practically all cases, however, the flasks to which boiled juice was added contained less tyrosine at the end of the run than did the controls. This was true even though the controls contained less tyrosine at the beginning than did the test solutions. The effect noticed must then be a true acceleration of enzyme action.

Effect of specific substances on tyrosinase activity

Our preliminary evidence (**13**) that potassium may act as an inhibitor of tyrosinase has not been supported by further tests. Addition of amounts of potassium chloride sufficient to equalize the potassium content of the added saps had no effect on the tyrosinase activity.

We have included tests of iron because of the results of Tinckler (**12**) and of Mader and Mader (**6**) indicating that this metal plays a rôle in discoloration after cooking. The addition to digests of either ferrous or ferric chloride in 0.01 per cent. concentration resulted in slight acceleration of tyrosinase activity in 0.1 M phosphate buffer at pH 7.0. As the activator in boiled juice has no effect in phosphate solutions, and since both forms of iron are active but the activator does not appear in the ash, iron is probably not the activator described above.

The addition of a small amount of catechol to a digest in borate buffer resulted in initial rapid melanin formation, as indicated by darkening, but the ultimate disappearance of tyrosine was not altered. It thus appears probable that the acceleration reported by Pugh (**9**) for both catechol and dihydroxyphenylalanine was temporary and followed by the action of an

inhibitor, as found by GRAUBARD and NELSON (4). As abnormal tubers contain high proportions of free amino acids it seemed possible that the observation of NOBUTANI (8) that these accelerate the oxidation of tyrosine might be significant in blackening after cooking. The addition of glycine in the proportion of one part to five of tyrosine in a borate-buffered digest practically inhibited the oxidation during the first several hours. At the end of 24 hours however the loss of tyrosine was the same as in the controls.

Summary

1. The tyrosinase activity of potato sap is affected by the particular buffer used in its determination. Phosphate accelerates the oxidation of tyrosine, while borates and phthalates inhibit the reaction. The inhibition is proportional to the concentration of the buffer.

2. The fact that the tyrosinase activity of tubers which discolor is higher than that of normal tubers was further substantiated. The difference in activity was apparent in several different buffers, in unbuffered solutions, and also at different pH values. Greater differentiation was apparent in either borate or unbuffered solutions than in phosphate buffers.

3. An activator of tyrosinase was found in the boiled sap of abnormal potatoes. It is not present in the ash and is lost in prolonged boiling or upon standing, and in dialysis. The activating effect was apparent in unbuffered solutions and in those buffered with borate, but non-existent in phosphate buffers. Since differentiation of the two types of potatoes is apparent even in phosphate buffers, the activator in boiled juice is not the only factor contributing to high tyrosinase activity in abnormal potatoes.

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CARBOHYDRATES OF WHEAT LEAVES[1]

G. KROTKOV

Introduction

Previous work on respiration and carbohydrate metabolism of wheat leaves on prolonged starvation (3) revealed the presence in these leaves of two different respiratory substrates, which were provisionally called the primary and the secondary substrates. The primary substrate has been found to be responsible for the production of CO_2 by leaves early in starvation, while its contributions in later stages were little or nothing. On the other hand the secondary substrate, which might or might not be used on the first day, was becoming with the progress of starvation of greater and greater importance, until in later stages either (as in some cases) all the CO_2 emitted by leaves was derived from it, or (as in others) the major portion.

It was shown in the same paper that the primary substrate was represented by sugars extracted from leaves by ethyl alcohol. While nothing definite was said on the nature of the secondary substrate it was suggested that this probably consisted of a variety of substances, some of which are of importance in protoplasmic organization. DELEANO (1) and YEMM (5) reported that proteins serve as the respiratory substrate in later stages of starvation in the leaves of grape and of barley. But before attention is definitely focused on proteins as the source of secondary substrate in wheat leaves, it ought to be determined whether or not some other carbohydrates, which might serve in this capacity, are present in leaves in sufficiently large amounts. NEWTON and BROWN (4), for example, reported that wheat leaves in later stages of maturity contain considerable amounts of pentosans. Such carbohydrates, if present, must remain in the leaf residue after alcoholic extraction, and they might exist there in the two following forms: (1) as hemicelluloses, (hydrolyzable by 1 per cent. H_2SO_4); (2) as part of a carbohydrate-protein complex, from which carbohydrates could be liberated on digestion of its protein fraction by trypsin. To test these two possibilities, the experiments reported below were performed.

Materials, methods, and results

EXPERIMENT 1

Seed of commercial Marquis wheat was sown in soil in a greenhouse on December 16, 1934, and eleven days later a sample of the plants was taken (sample 1). This consisted of plants cut after a whole day of illumination

[1] Part of the expenses of the experiments herein reported was defrayed by a grant from the Science Research Committee of Queen's University.

(3.4 hours of sunshine). A second sample (sample 2) was taken 18 hours later of plants which were kept in darkness after the taking of the first sample.

Following alcoholic extraction under a reflux condenser of minced plants, which consisted mainly of leaves, residues of both samples were digested either with unboiled or with boiled trypsin using 0.2 M Na_2HPO_4 as buffer. After digestion for 6 days at 37.5° C. filtrates from such digests were cleared in the usual way with basic lead acetate, and analyzed for their reducing power before and after inversion with HCl using the HAGEDORN-JENSEN method (**2**). Table I shows the results of analyses expressed as mg. of glucose liberated per 100 gm. of initial leaf fresh weight.

TABLE I

ANALYSES OF MARQUIS WHEAT LEAVES SHOWING MILLIGRAMS OF GLUCOSE LIBERATED PER 100 GRAMS OF FRESH WEIGHT

TESTS	TOTAL SUGARS	REDUCING SUGARS	INVERT SUGARS	INVERT SUGARS / REDUCING SUGARS
	mg.	*mg.*	*mg.*	
Sample 1				
Unboiled trypsin	0.105	0.066	0.039	0.59
Boiled trypsin	0.021	0.009	0.012	1.33
Difference	0.084	0.057	0.027	0.47
Sample 2				
Unboiled trypsin	0.143	0.078	0.065	0.83
Boiled trypsin	0.054	0.016	0.038	2.37
Difference	0.089	0.063	0.026	0.41

EXPERIMENT 2

Seed of a pure line of variety "Little Club" was sown in soil in a greenhouse during the spring of 1935. Leaves for all samples were always cut after a whole day of illumination, and the age of the plants for the samples 1, 2, and 3 was 13, 9, and 10 days respectively.

Following extraction with alcohol the leaf residues of all the three samples were digested with trypsin, and the digests so obtained were analyzed for their reducing power in the same way as has been described for experiment 1. Table II presents the results of analyses, expressed as percentage of initial leaf fresh weight.

Each sample of leaf residue following tryptic digestion was digested with 250 ml. of 1 per cent. H_2SO_4 for three hours in a water bath under a reflux condenser. The filtrates, obtained after such digestion, were neutralized with NaOH, cleared in the usual manner with basic lead acetate, and finally analyzed for their reducing power. Table III shows the results of analyses expressed as percentage of initial leaf fresh weight.

TABLE II

ANALYSES OF LITTLE CLUB WHEAT LEAVES SHOWING MILLIGRAMS OF GLUCOSE LIBERATED PER 100 GRAMS OF FRESH WEIGHT

TESTS	TOTAL SUGARS	REDUCING SUGARS	INVERT SUGARS	INVERT SUGARS / REDUCING SUGARS
	mg.	*mg.*	*mg.*	
Sample 1				
Unboiled trypsin	0.482	0.316	0.166	0.53
Boiled trypsin	0.189	0.052	0.137	2.63
Difference	0.293	0.264	0.029	0.11
Sample 2				
Unboiled trypsin	0.560	0.315	0.245	0.78
Boiled trypsin	0.286	0.080	0.206	2.57
Difference	0.274	0.235	0.039	0.17
Sample 3				
Unboiled trypsin	0.530	0.302	0.228	0.75
Boiled trypsin	0.271	0.095	0.176	1.85
Difference	0.259	0.207	0.052	0.25

Examination of tables I and II reveals that the tryptic leaf digests always contained some reducing substances, which were not removed from solutions after their clearing with basic lead acetate. Such substances are usually called sugars.[2]

Three explanations suggest themselves as to the source of reducing substances found in the digests with boiled trypsin: (1) They are some impuri-

TABLE III

COMPARISON OF REDUCING SUBSTANCES PRESENT IN TRYPTIC LEAF DIGESTS TREATED WITH ONE PER CENT. H_2SO_4. FIGURES GIVEN IN PERCENTAGE OF FRESH WEIGHT

	SAMPLE 1	SAMPLE 2	SAMPLE 3
	%	%	%
First half of sample after digestion with unboiled trypsin	0.482	0.560	0.530
Second half of sample after digestion with boiled trypsin	0.189	0.286	0.271
Difference (sugars liberated by trypsin)	0.293	0.274	0.259
First half of sample digested with one per cent. H_2SO_4	0.029	0.057	0.046
Second half of sample digested with one per cent. H_2SO_4	0.049	0.095	0.055
Difference	0.020	0.038	0.009
Percentage of sugars liberated by trypsin which is also liberated by one per cent. H_2SO_4	6.8	13.9	3.5

[2] Work in progress on the isolation and identification of carbohydrates from tryptic digests of leaves has shown that all the digests tested so far gave a very strong positive Molisch test.

ties from the trypsin solution; (2) they are the alcohol-soluble sugars not removed from leaves during alcoholic extraction; (3) they are the products of mild hydrolysis under the action of Na_2HPO_4 on some substances of the leaf residue.

In deciding which of the three suggestions is the most probable it is necessary to bear in mind the following facts: (1) ten ml. of the trypsin solution used for each digestion contained about 150 mg. of trypsin. It does not appear probable that this amount of trypsin, after clearing with lead, might contain the amounts of reducing impurities varying from 30 mg. to 61 mg. (calculated as glucose) as has been found by analysis. Moreover, if it does, then the absolute amounts of such impurities must be the same in all the digests, while they actually varied from one case to another. (2) Examination of the $\frac{\text{invert sugars}}{\text{reducing sugars}}$ ratios of the various digests reveals that this ratio is not constant. In digests with boiled trypsin it is always above unity. Again it is not very probable that reducing impurities from a trypsin solution should be characterized by such a ratio. (3) If the reducing substances present in digests with boiled trypsin are the alcohol-soluble sugars which escaped extraction, then their amounts are excessively high as becomes evident on their comparison with the values for sugars actually removed from leaves in alcoholic extraction. The amounts of total sugars extracted by alcohol in experiment 2 from the samples 1, 2, and 3 were 0.452, 0.361 and 0.716 per cent. respectively. It appears very doubtful that such a large proportion of alcohol-soluble sugars might escape extraction, since in no case was extraction carried out for less than 23 hours and since previous work (3) indicated that this time is ample to extract from wheat leaves the bulk of sugars.

Bearing the above-mentioned facts in mind, it appears more probable that reducing substances present in the digests with boiled trypsin are represented mainly by products of mild hydrolysis of some substances in leaf residue, while reducing impurities introduced in trypsin solution and alcohol-soluble sugars which escaped previous extraction might be found only in negligible amounts.

In experiment 1 the amounts of leaf residue used for the digestion with boiled and unboiled trypsin were not equal. On the assumption that reducing substances of the digests with boiled trypsin consist entirely of products of mild hydrolysis of some carbohydrate-yielding substances, the results of analysis have to be recalculated. Such corrected results are shown in table IV, expressed as percentage of fresh weight.

If we subtract the amounts of reducing substances in digests with boiled trypsin from those found in corresponding digests with unboiled trypsin, we obtain the amounts of sugars, which were liberated from leaves on diges-

TABLE IV

RECALCULATION OF ANALYSES OF LEAF RESIDUES WITH BOILED AND UNBOILED TRYPSIN. FIGURES GIVEN AS PERCENTAGE OF FRESH WEIGHT

TESTS	TOTAL SUGARS	REDUCING SUGARS	INVERT SUGARS	INVERT SUGARS / REDUCING SUGARS
	%	%	%	
Sample 1				
Unboiled trypsin	0.105	0.066	0.039	0.59
Boiled trypsin	0.038	0.015	0.023	1.53
Difference	0.067	0.051	0.016	0.31
Sample 2				
Unboiled trypsin	0.143	0.078	0.065	0.83
Boiled trypsin	0.056	0.016	0.040	2.50
Difference	0.087	0.062	0.025	0.40

tion with trypsin solution. These sugars might be liberated either from a carbohydrate-protein complex on digestion of its protein part with trypsin, or from some complex carbohydrates of leaves on their digestion with various polyases present as impurities in the trypsin solution. To explore these two possibilities the following experiment was carried out.

EXPERIMENT 3

On July 18, 1938, Marquis wheat was sown in soil in a greenhouse and nine days later a large number of plants was taken and extracted for 24 hours with 95 per cent. ethyl alcohol containing NH_3. Following extraction, the alcohol was filtered off, the leaf residue was dried and divided into two samples which were digested separately in a water bath for 3 hours with 1 per cent. H_2SO_4. Following such digestion, the leaf residue of each sample was filtered off, dried and divided into two equal parts. One part was digested with the unboiled and the other with the boiled trypsin. All the filtrates obtained from these digestions were cleared and analyzed for their reducing power.

The procedure used in this experiment was exactly the same as that used in the earlier experiments with only one exception: neutral, instead of basic lead acetate, was used for clearing. Table V shows the results of analyses expressed as percentage of initial leaf fresh weight.

Examination of table V reveals two facts: (1) H_2SO_4 digestion liberated considerably more reducing substances than had been found in the earlier experiments. This increase is probably caused partly by hydrolysis of some substances which in the earlier tests were hydrolyzed by the Na_2HPO_4 buffer, and partly to different environmental conditions existing during the growth of plants in this experiment. (2) Digests with the unboiled trypsin still showed an excess of reducing substances over corresponding digests with the

TABLE V

REDUCING POWER OF LEAF RESIDUES DIGESTED WITH 1 PER CENT. H_2SO_4 FOLLOWED BY BOILED AND UNBOILED TRYPSIN

TESTS	TOTAL SUGARS	REDUCING SUGARS	INVERT SUGARS	INVERT SUGARS / REDUCING SUGARS
	%	%	%	
Sample 1				
H_2SO_4, 1 per cent.	0.566			
Unboiled trypsin	0.276	0.165	0.111	0.672
Boiled trypsin	0.143	0.076	0.067	0.881
Difference	0.133	0.089	0.044	0.494
Sample 2				
H_2SO_4, 1 per cent.	0.518			
Unboiled trypsin	0.264	0.161	0.103	0.639
Boiled trypsin	0.136	0.080	0.057	0.712
Difference	0.128	0.081	0.046	0.567

boiled trypsin. Thus there are some substances in the leaf residue, which are broken down not by 1 per cent. H_2SO_4, but by trypsin, and from which are liberated reducing substances, called sugars according to our present-day terminology. This is equivalent to the statement that in the leaves there is a carbohydrate-protein complex, from which sugars can be liberated only by tryptic digestion of its protein component.

This conclusion does not exclude a possibility, that in experiments 1 and 2 some of the sugars found in the digests with the unboiled trypsin, were liberated there not by trypsin but by polyase impurities of the trypsin solution. Consequently in these experiments values for the sugars liberated from the carbohydrate-protein complex must be taken as the maximal values. In reality they might be lower.

It was shown previously (3) that starvation sequence in wheat leaves was brought to an end after a definite amount of the secondary substrate has been degraded. For 100 gm. of leaf fresh weight this amount is represented by 2748 ± 235 mg.[3] in CO_2 equivalents, or 1874 ± 160 mg. as glucose. On the other hand, the maximal amounts of the sugars which could be liberated from the leaf residues by trypsin, Na_2HPO_4 and 1 per cent. H_2SO_4 are represented in experiment 2 for the samples 1, 2, and 3 by 511, 617, and 576 mg. of glucose respectively. For both samples of experiment 1 such amounts probably would be even lower, considering that the values for the sugars liberated by 1 per cent. H_2SO_4 are small. We are forced, therefore, to conclude that leaf residues, following alcoholic extraction, do not contain easily hydrolyzable substances from which sugars could be liberated in amounts large enough to account for more than one-third of the secondary substrate. We must look, consequently, for some other substances besides carbohydrates

[3] Standard deviation has been computed with (N–1) degrees of freedom.

in our attempt to determine the nature of the materials forming the bulk of the secondary substrate.

Summary

1. Following alcoholic extraction of wheat leaves in the stage of early maturity the residue was found to liberate sugars after being hydrolyzed with Na_2HPO_4, digested with trypsin and, finally, after hydrolysis with 1 per cent. H_2SO_4.

2. While alcohol-soluble sugars, as well as those which are the products of mild hydrolysis with Na_2HPO_4 are characterized by the $\frac{\text{invert sugars}}{\text{reducing sugars}}$ ratio above unity, sugars liberated on tryptic digestion have a preponderance of reducing over invert sugars.

3. It has been shown that the union between the component parts of the carbohydrate-protein complex is of such a nature, that it is not broken down on digestion with 1 per cent. H_2SO_4, and that sugars can be liberated from such a complex only after tryptic digestion of its protein fraction.

4. Present work failed to reveal any substances in leaf residue from which sugars can be easily liberated in sufficient amounts to account for the entire secondary respiratory substrate.

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STIMULATION OF GERMINATION OF DORMANT LETTUCE SEED BY SULPHUR COMPOUNDS

ROSS C. THOMPSON AND WILLIAM F. KOSAR

(WITH ONE FIGURE)

Numerous agents have been reported as effective in promoting germination of dormant lettuce seed. BORTHWICK and ROBBINS (**1**) found that the percentage of lettuce seed germinating at temperatures above 25° C. is increased by high oxygen pressure. THORNTON (**17**) reported that good germination was obtained at temperatures as high as 35° C. if the surrounding atmosphere contained from 40 to 80 per cent. of carbon dioxide. SHUCK (**14, 15**), FLINT (**7**), and FLINT and MCALISTER (**8**) have reported on the influence of light on the germination of dormant lettuce seed.

DENNY (**2, 3**) and MILLER (**13**) have studied the influence of certain sulphur compounds on the breaking of dormancy in the potato tuber. That certain sulphur compounds are also effective in breaking dormancy in lettuce seed was reported by the writers (**16**). The present paper is a more complete report of studies made on the influence of chemicals in stimulating germination of lettuce seed under conditions that result in poor germination if the seed is not given some special treatment.

Material and methods

In making these studies, aqueous solutions of the chemicals were used as media in which the seeds were germinated. The tests were all made in 95-mm. Petri dishes. A piece of filter paper was first placed on the bottom of each dish. Fifty seeds were scattered on the paper and two ml. of the solution to be tested were added by means of a measuring pipette. The dishes were covered and placed immediately in a germinator in darkness at 24° to 26° C. The dishes were removed and the germinated seeds counted after 72 hours. Each test was run in quadruplicate.

The chemicals that have been tested for their influence on the germination of lettuce seed include acetamide, thioacetamide, allyl urea, allyl thiourea, ammonium sulphate, ammonium thiocyanate, asparagin, calcium thiocyanate, potassium thiocyanate, potassium cyanide, potassium ferricyanide, potassium ferrocyanide, semi-carbazide hydrochloride, thio-semicarbazide, sodium nitrate, sodium thiocyanate, sulphuric acid, urea, thiourea, ammonium nitrite, hydrazine, and sulphanilic acid.

Only chemically-pure materials were used. Each chemical was first tested in various strengths of aqueous solution to determine the concentration giving the highest percentage of germination. A few of the materials were injurious at concentrations as low as 0.1 per cent. Nearly all were injurious in 1.0 per cent. solutions.

Six of the chemicals tested gave very marked increases in germination over the untreated checks. The chemicals having a marked stimulating influence and their optimum concentration are as follows: thioacetamide, 0.4 per cent., and thiourea, allyl thiourea, thio-semicarbazide, ammonium thiocyanate, and potassium thiocyanate, 0.5 per cent. Sodium thiocyanate and calcium thiocyanate gave some significant increase in germination on some lots of seed but neither of these had the stimulating influence exhibited by the six above-mentioned chemicals. All of the other chemicals retarded germination in most cases except in very dilute concentrations.

Experimentation

Relative effectiveness of chemicals in stimulating germination

After the optimum concentration of the chemicals had been determined, a series of tests, hereafter known as series I, was made, using the six most effective chemicals—allyl thiourea, thioacetamide, thiourea, thio-semicarbazide, ammonium thiocyanate, and potassium thiocyanate. The results from this series of tests are given in tables I and II.

TABLE I

Germination of chemically-treated seed of four lettuce seed stocks
Series I

Treatment	Germination in percentage of 200 seeds in 4 replications				Mean
	Seed stock number				
	1	2	3	4	
	%	%	%	%	%
Check*	79.0	4.0	0.0	100.0	45.75
Thiourea	99.5	78.0	18.0	100.0	73.86
Thio-semicarbazide	93.0	55.0	30.0	98.0	69.00
Thioacetamide	87.0	70.5	0.0	96.0	63.38
Allyl thiourea	67.5	63.0	0.5	76.0	51.88
Ammonium thiocyanate	51.0	47.5	0.5	95.5	48.63
Potassium thiocyanate	45.5	15.0	0.5	94.0	38.63
Mean	74.64	47.71	7.0	94.14	

* Differences required to show significance are as follows: treatments, 5.58 per cent.; stocks, 4.24 per cent.

Four lots of seed were used. Three of these showed some degree of dormancy when untreated and the fourth was a non-dormant lot giving a check test of 100 per cent. germination on filter paper moistened with water. The three dormant lots were numbered 1, 2, and 3, and the non-dormant stock no. 4. The dormant lots gave check germination tests of 79.0, 4.0, and 0.0 per cent., respectively. The non-dormant lot was included to determine the influence of the chemicals on seed not normally dormant.

TABLE II

ANALYSIS OF VARIANCE OF DATA FROM SERIES I, TESTING THE RELATIVE INFLUENCE OF SIX CHEMICALS IN STIMULATING GERMINATION OF LETTUCE SEED

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE
Stocks	3	9,984.72
Treatments	6	603.68
Replications	3	50.48
Stocks × treatments	18	275.06
Error	81	15.61
Total	111	

The different seed stocks varied greatly in their response to treatments, as indicated by the large mean square value for stocks in table II.

The highly significant variance for interaction between stocks and treatments shows definite differences among stocks in response to a given treatment. Variance due to treatment alone is not significantly greater than interaction so it is not possible to generalize with reference to the relative effectiveness of treatment for any specific lot of seed. Variance due to stocks, however, is significantly greater than interaction, showing that these stocks, in general, maintain their relative responsiveness to individual treatments.

IMPORTANCE OF SULPHUR IN THE COMPOUND

Of the six chemicals having a marked stimulating influence on germination, two are inorganic—ammonium thiocyanate and potassium thiocyanate—and four, allyl thiourea, thioacetamide, thio-semicarbazide, and thiourea, are organic. The compounds in the former group have the CNS linkage in common and those in the latter group all have the amino NH_2 linked to the CS. All six have sulphur in common. The importance of sulphur in the compound is indicated by the results obtained from series II of tests in which the thio-compounds were tested with similar compounds in which sulphur is absent. In this series, thioacetamide was compared with acetamide, thiourea with urea, allyl thiourea with allyl urea, and potassium thiocyanate with potassium cyanide. In each case the most effective concentration of the chemical was used. No ammonium cyanide or semi-carbazide was available for testing with ammonium thiocyanate and thio-semicarbazide. The results of this series of tests are presented in tables III and IV. In each of the four comparisons made, the absence of sulphur from the compound not only resulted in no stimulation of germination but in every case retarded germination in concentrations of 0.1 per cent. or higher.

These results (tables III and IV) show clearly the need of sulphur in the compound for its stimulating influence on germination. The presence of sulphur in the compound is not the only condition necessary, as is shown by the failure of ammonium sulphate, calcium sulphate, potassium sulphate,

TABLE III

GERMINATION OF LETTUCE SEED TREATED WITH CHEMICALS WITH AND WITHOUT SULPHUR
SERIES II

TREATMENTS*	GERMINATION IN PERCENTAGE (200 SEEDS IN 4 REPLICATIONS)			MEAN
	SEED STOCK NUMBER			
	1	2	3	
	%	%	%	%
Thiourea	96.0	56.5	8.5	53.67
Urea	20.5	0.0	0.0	6.83
Allyl thiourea	89.5	39.0	0.5	43.00
Allyl urea	8.0	0.0	0.0	2.67
Thio-acetamide	96.0	48.0	0.0	48.17
Acetamide	18.5	0.0	1.5	8.33
Potassium thiocyanate	84.0	5.0	0.0	29.67
Potassium cyanide	19.0	1.0	0.5	6.83
Check	22.5	1.0	2.0	8.50
Mean	51.00	16.78	1.44	

* Differences required for significance are as follows: treatments, 3.54 per cent.; stocks, 2.32 per cent.

and sulphuric acid in any concentration between 0.1 and 1.0 per cent. to promote germination. The presence of C, N, and S in some specific linkage is apparently necessary.

TABLE IV

ANALYSIS OF VARIANCE OF DATA FROM SERIES II, TESTING THE INFLUENCE OF SULPHUR IN THE COMPOUND

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE
Treatments	8	1,267.40
Stocks	2	5,793.03
Replications	3	4.06
Treatments × stocks	16	404.63
Error	78	4.71
Total	107	

Although the interaction between stocks and treatments is significant with reference to error, the variances due to stocks and treatments are both significantly greater than interaction. Thus, the influence of treatments and the behavior of stocks were generally quite consistent.

Discussion

Many tests in which chemicals were used show that thiourea is the most generally effective material in promoting germination of dormant lettuce seed. Thiourea in the 0.5 per cent. concentration resulted in some stimulation of germination in every lot of dormant seed on which it has been used.

In some cases it has given nearly 100 per cent. germination on seed that was completely dormant in water under the same conditions of temperature and light.

The other five compounds—allyl thiourea, ammonium thiocyanate, potassium thiocyanate, thio-semicarbazide, and thioacetamide—were more variable in their effect on different lots of dormant seed. On some lots their stimulating effect was very great, while other lots were not stimulated by the chemical.

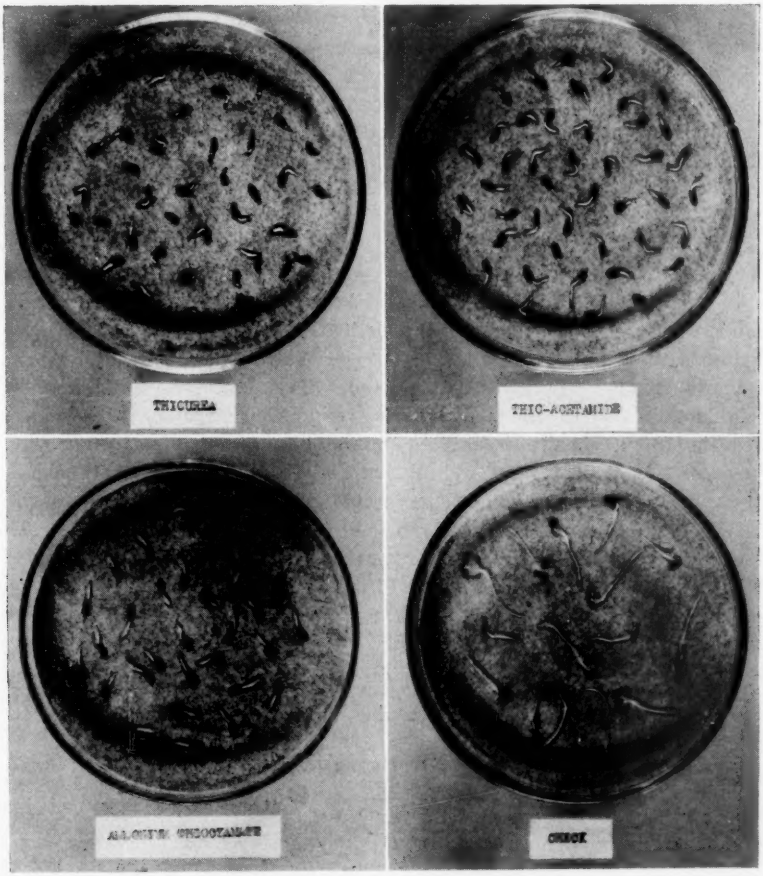

FIG. 1. Sprouted lettuce seed after 72 hours in darkness at 25° C. A, in 0.5 per cent. thiourea; B, in 0.4 per cent. thioacetamide; C, in 0.5 per cent. ammonium thiocyanate; D, in tap water.

In order of general effectiveness in stimulating germination on all of the various seed samples studied, the compounds rank in the following descending order: thiourea, thio-semicarbazide, thioacetamide, allyl thiourea, ammonium thiocyanate, and potassium thiocyanate. The last two named materials have a very marked influence on some lots of seed but on many samples they have little effect. Potassium thiocyanate is the most toxic and causes considerable injury to the radicle in some cases.

All of these compounds except ammonium thiocyanate have a very marked retarding effect on hypocotyl and radicle elongation and on root hair development. With ammonium thiocyanate, radicle and hypocotyl elongation and root hair development are about normal except for a slightly slower rate of growth. The type of growth resulting from the use of thiourea, thioacetamide, thio-semicarbazide, allyl thiourea, and potassium thiocyanate is very similar to that reported by BORTHWICK and ROBBINS (1) as resulting when lettuce seed is germinated under high oxygen pressure. The growth-inhibiting effect of some of the compounds used is shown in figure 1.

With the exception of this semi-carbazide, the inhibitory effect of these chemicals is not permanent. When the seeds were removed from the chemical solutions as soon as they had germinated, and planted in soil, the treated lots emerged from the soil and developed as rapidly as the seed germinated in water, with the exception of those germinated in thio-semicarbazide. Seed treated with this material failed to emerge. The rôle of these sulphur compounds in the germination of lettuce seed is not known, and no attempt has been made in the present studies to determine their function in the process of germination. DRYERRE (6), HANES and BARKER (9, 10), JOHNSON and WORMALL (11), DENNY (4, 5), MILLER (13), and others have studied the effect of certain of the cyanides and cyanates on enzyme activity. Enzyme activity has been found to increase in the presence of KCN and KSCN. The results reported on the effect of these materials on enzyme activity suggest that the sulphur compounds found to have a stimulating influence on the germination of dormant lettuce seed may have some similar function in the process of germination of lettuce seed.

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SOME FACTORS CONTRIBUTING TO TOMATO PUFFING[1]

J. J. TAUBENHAUS[2] AND G. E. ALTSTATT

Tomato puffing is a common fruit defect causing serious annual losses in Texas. The trouble is known by several names, such as "tomato puffs," "tomato pops," "puffy tomatoes," "puffs," and "pockets." The term tomato puffing, as is now generally used by tomato growers wherever the defect occurs, is preferred.

In 1895 PRICE and NESS (**6**) of the Texas Agricultural Experiment Station gave the first clear description of tomato puffing of the variety "Terra cotta" in which they seem to have accepted puffing as a varietal characteristic. In 1926 LESLEY and ROSA (**5**) recognized puffing as a defect of some tomato crosses grown at Riverside, California. WEBER and RAMSEY (**15**) found the same trouble causing serious damage in Florida-grown tomatoes. TAUBENHAUS (**7**) in 1929, reported some preliminary observations on puffed tomatoes. Since then, TRAUB *et al.* (**13, 14**) and YARNELL *et al.* (**17** to **25**) and CORNS (**1**) have added much to our knowledge of this trouble.

The most typical symptom of a puffed tomato is its angular appearance, with slightly indented walls which yield readily to the least pressure. In some varieties the flatness of the sides is not so conspicuous, but the light weight and the hollow "feel" of the fruit is a sure indication of puffing. A severely puffed fruit upon being sliced shows a partial to complete abortion of the placental tissues, which accounts for the hollow interior. Sometimes the locular walls are much proliferated and hardened. Tomatoes in which the hollow cavity is negligible are classified as no. 1 fruits and marketable, while fruits with prominent cavities are classed as culls.

To determine the prevalence of tomato puffing in the United States in 1933, a questionnaire was sent to plant pathologists and horticulturists in every state as well as in Alaska, Hawaii, Puerto Rico, and the Virgin Islands. The replies received reported tomato puffing of field-grown tomatoes in Alabama, Arkansas, California, Louisiana, Mississippi, and Texas, but only of greenhouse-grown tomatoes in Illinois and Michigan. During 1924, field losses were estimated at 14 per cent.; in 1925, 15 per cent.; in 1926, 20 per cent.; in 1927, 2 per cent.; in 1928 and 1929, 8 per cent.; in

[1] Published with the approval of the Director as contribution no. 440, Technical Series, of the Texas Agricultural Experiment Station.

[2] Deceased. This paper is one of the last written by the late Dr. J. J. TAUBENHAUS. It represents several years of experimentation and observation. One will note the habit of making large numbers of samples and cultures, so typical of his work. Not a hundred cultures, but two thousand; not a hundred fruits, but many bushels of them were examined. Since the original manuscript left his hands, only the changes suggested by readers of the manuscript have been made by the junior author.

1930, 14 per cent. of the commercial Texas crop. Tomato growers and packers in Texas generally agree that losses from tomato puffing may vary not only from year to year, but even in the same season, depending on weather conditions. After a heavy or prolonged rainy spell greater culling is necessitated in both field and packing sheds, due to unusual increases in puffed tomatoes, while during dry weather, tomato puffing, although present, is generally unimportant.

It is generally believed by growers that puffing becomes noticeable only when the tomato fruits are fully developed and just before they begin to color. In order to determine whether puffing is a defect of the maturing fruit only, a systematic survey (**8**) was carried out during 1930 at 18 different locations in Texas. Tomato fruits in all stages of development were cut and the degree of puffing recorded. It was found that tomato puffing in its earliest and severest occurrence was just as prevalent in young embryonic fruit, even only 1.0 to 2.5 mm. in diameter, as in fully developed and maturing tomatoes.

As to susceptibility, YARNELL (**17, 18, 22**) found considerable variation among different tomato varieties; the Marglobe was the most susceptible, while the John Baer was the most resistant. The writers found that the John Baer variety was very susceptible to tomato puffing when grown under irrigation in the Winter Garden section of Texas. Soil moisture conditions seem to affect the susceptibility of a variety because during 1930 the Marglobe, a highly susceptible variety, showed 19 per cent. affected fruit when grown near Troup, 22 per cent. at Weslaco, and 46 per cent. when grown near Bryan, Texas.

Opinion as to the cause of puffing differs greatly. Some growers claim that the trouble is favored by the use of excessive amounts of fertilizer, particularly nitrate of soda, while others believe moisture to be the cause, since the greatest losses occur in seasons of continuous precipitation or after a heavy rain following a prolonged dry spell. CORNS (**1**) found that in greenhouse studies puffing was associated with poor pollination, but that pollination was not a factor under field conditions. FOSTER and TATMAN (**2**) after 5 years of study on puffing of tomatoes in the greenhouse, concluded that growth-limiting factors such as soil moisture, soil nutrients, and air temperature or combinations of these, may interfere with the normal development of the fruits.

During 1928 and 1929, about 2000 plate cultures on potato-dextrose agar were made from tissue of lightly to severely puffed tomato fruit, and in not a single instance was an infectious organism recovered. With no indication of a causal organism, one might first suspect that tomato puffing is either of a physiological nature or is induced by some virus. In order to determine whether tomato puffing is of a virus nature (**8**) Marglobe tomato

seeds were obtained during 1929 from apparently normal fruits. The next spring (1930) they were disinfected for 5 minutes in 1:2000 mercuric chloride, and planted in insect-proof cages at College Station. This test was repeated in 1931. During both years the plants in all the cages were hand pollinated to insure a crop of tomato fruits. About the middle of the growing season the plants in some of the cages were inoculated with freshly extracted, filtered juice obtained from freshly gathered, severely puffed tomatoes. This juice was passed through a Berkfeld filter, and used as inoculum, being either rubbed into the leaves and stems with a sterile brush, or injected with a hypodermic syringe. Check plants were rubbed or injected with distilled sterilized water. A small number of the inoculated and uninoculated plants in the cages showed mosaic. Puffed fruits occurred in all of the cages and on all the tomato plants, whether inoculated or not and whether naturally infected by mosaic or not. This suggests that tomato puffing is not caused by a virus, as otherwise the uninoculated and the non-mosaic-infected plants in the check cages should have produced normal tomatoes, which was not the case.

In 1932 (**10**) seed of Marglobe tomatoes were obtained from Wethersfield, Connecticut, where puffing is not known to occur. These seeds were surface sterilized for 5 min. in a 1:2000 mercuric chloride solution, planted in insect-proof cages at College Station, and thinned to a stand of nine plants per cage. The blossoms of all the caged plants were hand pollinated. A high percentage of puffed fruit occurred on all of the plants, whether these plants were naturally infected by mosaic or not. These results further suggest that puffing is not caused by any seed-borne virus, but they point to the probability of some environmental factors as the cause.

Seed from the same lot was surface sterilized and planted in steam-sterilized and unsterilized Lufkin fine sandy loam. All plants were dusted with insecticides frequently to keep them free of insects. Puffed fruit developed on all plants, regardless of soil treatment, indicating that puffing is probably not of an insect-borne virus nature.

If tomato puffing is not caused by a pathogenic organism or by a virus, then is it a trouble brought about by certain factors of environment acting on particular hereditary complexes? Several factors have thus far been tested including soil acidity, fertilizers, soil moisture, geographical location, and the effects of other diseases upon puffing.

During 1933 (**12**), Marglobe tomato plants were grown at College Station, in wooden containers which were filled with different known soil type materials and of known degrees of acidity. Container 1 was filled with Susquehanna fine sandy loam having a pH of 5.5; container 2 was filled with Tabor fine sandy loam with a pH of 6.7; container 3 was filled with Kirvin fine sandy loam with a pH of 7.1; and container 4 was filled with

Houston black clay soil with a pH of 7.7. No significant difference in the percentage of puffed fruits occurred in the different soil types used. In other experiments, earthenware cylinders filled with Lufkin fine sandy loam were adjusted to acidities varying from pH 4.9 to pH 8.5 A total of 4 cylinders were used for each pH adjustment and a single tomato plant was planted in each cylinder. There appeared to be no correlation between the soil acidity and the amount of puffed fruit in each cylinder.

FRIEND (**3, 4**) found that differential fertilizer treatments had no significant influence on puffing. YARNELL *et al.* (**17** to **25**), on the other hand, stated: "Differential fertilizer treatments did not affect the amount of puffing in the Lower Rio Grande Valley, although a difference was found at College Station," which might be explained by differences in soil fertility. The same authors also found that several varieties of tomatoes always produced less puff when fertilized with a 6–12–6 fertilizer.

In 1933, super-phosphate, sulphate of potash, and nitrate of soda in definite amounts and in various combinations were added to Lufkin fine sandy loam soil in galvanized cylinders which were set in the ground at College Station, Texas. A single Marglobe tomato plant was planted in each cylinder and regularly watered twice a week from June 1 to November 1, except during rainy weather. Puffed fruits were found on all plants irrespective of the fertilizer added to the soil.

The possible relationship of soil moisture to tomato puffing has been long suspected, as previously indicated by Texas and Florida tomato growers. Although the literature on this relationship is somewhat conflicting, the trend of experiments by various workers indicates a correlation of the amount of puffed fruit with the amount of moisture present in the soil. FRIEND (**3, 4**) found that the yield was greater and the percentage of puffy fruit lessened from plants receiving greater amounts of water at frequent intervals, whereas YARNELL (**17** to **25**) found: "In general the amount of puffing is greater where there is more available moisture." WOOD (**16**) found that tomatoes grown without irrigation showed considerably less puffing than those grown with irrigation. This was especially true with the varieties Pritchard and Marglobe. During 1930, observations were made at Arp, Tyler, and Prairie View of tomatoes grown on dry upland soil, moist lowland soil, and under conditions of overhead irrigation. Lower percentages of severely puffed fruit occurred on plants growing on upland dry tight soil, while high percentages of puffed fruit were found on plants growing in seepy lowlands or on plants grown directly under irrigation. In 1932 and 1933 (**10**), Marglobe tomatoes were grown at College Station in plats receiving weekly irrigations and in plats without irrigation. The greatest percentage of normal tomatoes was produced on individual plants growing without irrigation. These experiments further indicate the relationship of soil moisture to tomato puffing.

It has been prevously mentioned that there are certain sections in the United States where tomato puffing is unknown. Tomato seeds were obtained in 1932 from these sections. For comparison, tomato seed was also obtained from plants grown in Florida and Texas where annual losses from tomato puffing are heavy. The plants from each of these sources of seed were divided into two lots, eight of which were grown under irrigation and eight without irrigation. Irrespective of origin of the seed, puffing occurred in all strains and varieties, particularly when grown under irrigation. These studies suggest that puffing may be caused by various temperature, soil, or moisture conditions or other factors peculiar to some of the southern states. Tomato seed from sections where tomato puffing never occurs did not produce plants entirely free from affected fruit.

It was thought that the presence of other diseases on the tomato plant might be a factor in affecting the amount of puffed fruit. TRAUB (**13**) observed that mosaic-infected tomato plants produced more puffed fruits than normal plants. In our inoculation experiments tomato puffing appeared equally prevalent irrespective of natural mosaic infection. Tomato fields were examined in 1930, 1931, and 1932 to determine whether there was any correlation between the number of puffed tomato fruits per plant, and the infection of that plant by certain other organisms. Records were made of mature plants affected by mosaic (virus), Fusarium wilt (*F. lycopersici*), Septoria leaf spot (*S. lycopersici*), Alternaria leaf spot (*A. solani*), Cladosporium leaf mold (*C. fulvum*), and bacterial canker (*Aplanobacter michiganense*). Puffing occurred equally as often on plants infected with these diseases as on those which were non-infected. YARNELL *et al.* (**25**) found no relationship between the amount of puffing and injury to the plants by *Sclerotium rolfsii*. The same writers also observed that there was no association between blossom-end rot, a physiological disease caused by abnormal water relations, and the amount of puffing.

Summary

Tomato puffing is a fruit defect causing annual losses of 8 to 15 per cent. of the tomato crop in Texas. Affected fruits are light in weight, angular, and flat-sided, and more or less hollow. Puffing begins in the embryonic stage and progresses with the development of the fruit; the symptoms, however, are more prominent in fully developed tomatoes.

The cause of puffing is still unknown. The studies here reported seem to show that it is not caused by a microorganism nor a virus. Tomato puffing does not appear to be seed- or soil-borne, nor is it influenced by other diseases attacking the same plant, nor by soil acidity. The defect is influenced by soil moisture and probably by certain fertilizers, as well as some environmental

conditions. Irrigated plants produced a higher percentage of puffed fruits than did plants grown without irrigation.

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ELECTRODIALYSIS OF PEA SEEDS

WENDELL R. MULLISON

(WITH THREE FIGURES)

Introduction

Electrodialysis differs from ordinary dialysis in that an electric current is passed through the dialyzing cell and the impressed electrical potential hastens the removal of ions from colloidal material. Consequently, electrolytes are removed faster and to a greater degree than by ordinary dialysis.

In the last twenty-five years electrodialysis has been used extensively in the study of the composition and purification of biological materials. PAULI (**4**) was the first worker to use this technique in the purification of proteins, whereas now it is a routine procedure. It has been applied extensively in the study of soil colloids and in general is the best method for the purification of lyophilic colloids.

The technique has been used recently in other ways. MOORE, REEVES, and HIXON (**2**) electrodialyzed normal and abnormal apple tissue in an attempt to determine the cause of spotting which occurs in Jonathan apples during cold storage. NELLER (**3**) also used electrodialysis in a study of apple tissue. More recently COOPER, PADEN, and SMITH (**1**) used the same method in treating fresh samples of cotton, corn, and soy bean tissue in order to determine the ease of removal of various cations.

The present study was undertaken in order to determine the effect of electrodialysis upon the subsequent development of seedlings.

Methods and material

The apparatus consisted of a water-tight wooden box so constructed that it could be divided into three compartments upon the insertion of two cellophane partitions. The inside of the box was thoroughly impregnated with paraffin to prevent the withdrawal of any electrolytes from the wood. The cellophane used was Du Pont's non-waterproofed no. 300. The electrodes were graphite plates and the source of current was an 80-volt Edison storage battery. The temperature of the apparatus was kept below 30° C. by means of running cold water through a glass-tube cooling system.

Pea seeds of the Alaska variety were used. They were placed under germinating conditions for twelve hours before being dialyzed. The seeds were then divided into two groups of 75 or 100 each. One group was placed in the middle compartment of the electrodialyzing cell and 100 ml. of distilled water was then added to each compartment. The control group was placed in a beaker containing the same amount of distilled water and left submerged for a period equal to the time of dialysis.

Experimentation

PETRI DISH CULTURES.—Sixteen hundred seeds were used during various repetitions of this work. Lots of one hundred seeds were used. Six seeds of each lot were removed from the dialysis chamber every fifteen minutes up to one hour, and then at intervals of thirty minutes up to three hours. The remaining seeds were discarded, having served the purpose of increasing the amount of current passing through the cell. As each sample and its control were removed they were placed on filter paper, moistened with distilled water, and placed in Petri dishes to germinate. Those seeds which had been dialyzed longer than one hour had a yellowish cast; those dialyzed for three hours had this discoloration to a pronounced degree. The percentage of germination varied from about 100 per cent. in the controls to about 70 per cent. for the seeds dialyzed for one hour; with three hours of continuous dialysis the germination rate was reduced to about 20 per cent.

After germination, the differences were manifested in other ways. Seeds

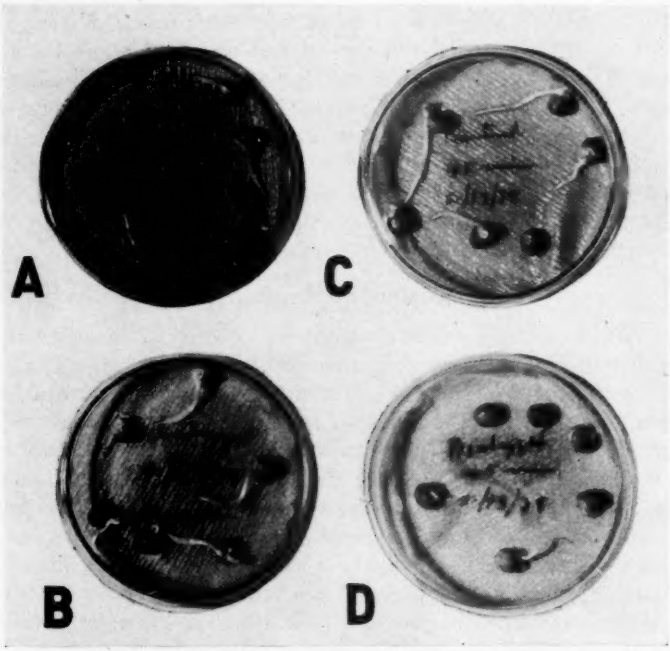

FIG. 1. Growth of pea following electrodialysis of the seeds; age 1 day. A. Control; soaked 15 minutes without dialysis. B. Electrodialyzed 15 minutes. C. Control; soaked 45 minutes without dialysis. D. Electrodialyzed 45 minutes.

dialyzed for periods of more than one hour showed a slower rate of germination, a slower rate of growth that often stopped entirely after a short time, and a marked susceptibility to infection from fungi (see figs. 1 and 2).

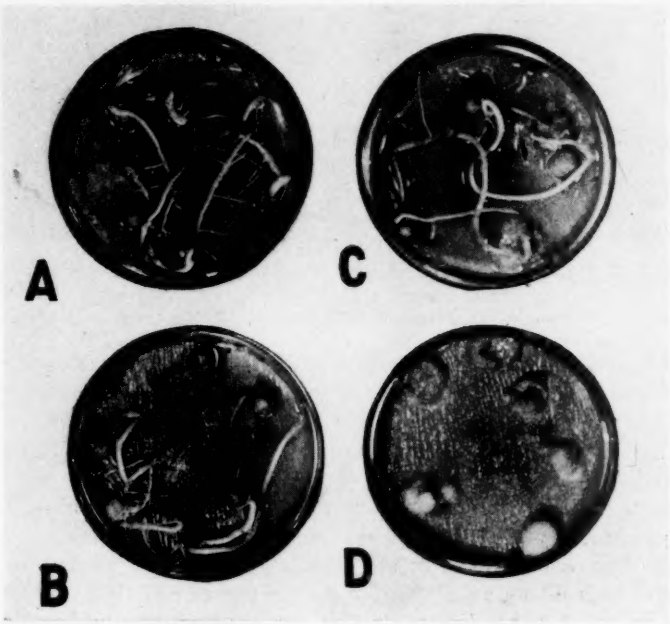

FIG. 2. Growth of pea seedlings from electrodialyzed seeds; age 7 days. A. Control; soaked 1 hour without dialysis. B. Electrodialyzed 1 hour. C. Control; soaked 3 hours without dialysis. D. Electrodialyzed 3 hours.

Indeed, the latter was so marked that after a few days' germination the Petri dishes containing the dialyzed seeds could be identified by the heavy growth of mold whereas the controls were rarely affected. Root hair development was greatly retarded in those seeds which had been dialyzed for the longer periods of time. They were shorter in length and fewer in number.

SAND AND SOIL CULTURES.—The same procedure was followed as in the previous experiments. In this case, ten seeds were removed from the dialyzer at the end of the first, second, third, and sixth hours. They were watered with tap water. The results at the end of three weeks closely approximated the results obtained from germinating the seeds in Petri dishes. In addition to the effect on germination, there was a progressive, decreasing gradation in size from the controls to the seeds dialyzed for three hours. All of those dialyzed for six hours were killed (fig. 3).

FIG. 3. Effect of electrodialysis on peas grown in sand with tap water; age, 3 weeks. A. Control; soaked 6 hours without dialysis. B. Electrodialyzed 1 hour. C. Electrodialyzed 2 hours. D. Electrodialyzed 3 hours. E. Electrodialyzed 6 hours.

Other seeds were then similarly treated except that they were planted in soil. The percentage of germination was affected as in all the other cases. The rate of development was at first slowed up but after three or four weeks the surviving dialyzed plants apparently recovered entirely and could not be distinguished from the controls.

EFFECTS OF ELECTRODIALYSIS ON DRY WEIGHT AND ASH.—The air-dry weight of lots of seventy-five pea seeds was recorded. Here the procedure differed a little from that followed in the preceding experiments in that all of the seeds of each lot were dialyzed for the same length of time. Various lots were dialyzed for periods of three, four, five, seven, and twelve hours. After

TABLE I

DRY WEIGHT AND ASH OF ELECTRODIALYZED SEEDS

TIME	TREATMENT	AIR-DRY WEIGHT	OVEN-DRY WEIGHT	ASH WEIGHT	PERCENTAGE ASH*
3 hr.	control	14.343	13.170	0.377	2.63
	dialyzed	14.386	12.870	0.232	1.61
4 hr.	control	14.213	13.143	0.363	2.56
	dialyzed	14.264	13.015	0.220	1.54
5 hr.	control	14.429	13.178	0.344	2.38
	dialyzed	15.389	12.670	0.139	0.914
7 hr.	control	14.366	13.396	0.322	2.24
	dialyzed	15.079	13.094	0.142	0.942†
12 hr.	control	14.491	13.346	0.146	1.09
	dialyzed	15.034	12.282	0.071	0.473

* Based on air dry weight.
† Reason for this discrepancy not known.

treatment, the seeds were dried at 100° C. and weighed. They were then ashed in platinum crucibles and the ash weight was determined. Results are shown in the following table.

It is evident from table I that a large amount of material may be lost through simply soaking the seeds. At the end of twelve hours of soaking the control seeds had lost about 60 per cent. of their original ash weight. This loss, however, did not affect the viability of the seeds as judged by their germination. The seeds which had been electrodialyzed lost much more material than did the controls by mere soaking. Comparing the electrodialyzed seeds with the controls on a basis of their ash weight the dialyzed seeds retained approximately 40 to 60 per cent. less ash than did the soaked controls. These figures do not take into consideration the slight differences in the original weight of the two lots of seeds which, however, would not materially change the above values.

Conclusion

It is evident from the foregoing data that electrodialysis has a detrimental effect upon percentage of germination, resistance to infection, and subsequent development of seedlings. If the treatment is not too long-continued, the plant is apparently able to replace the lost electrolytic material, at least in part, with that obtained from the soil. If the seeds are electrodialyzed for too long a period, they lose their viability.

The writer expresses his appreciation to Dr. C. A. SHULL for his interest in this study.

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INTERPRETATION OF COMPARATIVE GROWTHS OF FUNGAL COLONIES GROWING ON DIFFERENT SOLID SUBSTRATA

CLAIR L. WORLEY

(WITH TWO FIGURES)

A survey of the available literature in the fields of plant physiology, mycology, and plant pathology clearly indicate that there is a controversy regarding the most accurate method of interpreting the growth extent of a fungous mat on solid nutrient media. Accuracy is particularly important when comparisons are being made of the effects of media with and without various concentrations of growth promoting and growth inhibiting substances. A like controversy has occurred as to the best method to be applied in an effort to interpret accurately the growth curve for a specific mat over a period of several consecutive days.

Two distinct viewpoints have been apparent. The one group holds that in the measurement of colony growth a comparison of the radii is a better criterion of actual growth rate, while the others maintain the ratio of the respective areas is the better. Seldom has a worker seen the desirability of including both types of calculations and of drawing conclusions which would be of significance regardless of the method employed.

In presenting a third method of calculation, it is not the purpose of this paper to magnify this controversy, but rather to present the various techniques and the arguments for and against each.

The various methods and their interrelationships can be derived from figure 1. Figure 1–K represents the control mat and figure 1–T the test mat. In the determination of the growth curves for a given fungous mat, figure 1–T would represent a later stage of growth for the mat of figure 1–K.

The various methods which may be applied to the problem concerned are given in outline form below. All letters included refer to the same letters in figure 1.

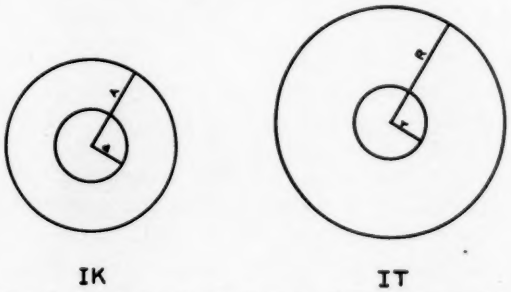

FIG. 1. Diagram of fungal mats. Explanation in text.

TYPE I. Comparison of radii (or diameters):

I–a. The length of each radius (or diameter) for the fungous mat on the control and on the test medium is plotted against time, and the several curves are compared.

I–b. A ratio is set up between the radii of the control and the tests at each measurement. This is the resultant of I–a. The value of the ratio, in all cases, becomes $R_0 = R/A$.[1]

I–c. The increases of radii are plotted as a curve against time. A comparison is then made between the curve for the control and those for the tests.

I–d. The ratio of the increments of the respective radii claims the greater number of advocates. The value for this ratio is $DR_0 = (R-r)/(A-a)$.

TYPE II. Comparison of areas:

II–a. The total areas for the test mat and the control mat are plotted in reference to time, and the resultant curves are compared.

II–b. A ratio is established between the total areas; that is, the ratio of πR^2 to πA^2. Therefore $A_0 = R^2/A^2$, which is equal to R_0^2.

II–c. The increases of areas are plotted as a curve against time. A comparison is then made between the curve for the control and those for the tests.

II–d. The ratio of the increases of the respective areas claims the next largest group of exponents. This ratio becomes $DA_0 = (\pi R^2 - \pi r^2)/(\pi A^2 - \pi a^2)$; that is $DA_0 = (R^2 - r^2)/(A^2 - a^2)$.

TYPE III. Apparently, the method offered in this section is new and the arguments in its favor are presented in the following paragraphs. The method takes into consideration the numerous points of growth from the original disk and the area that each covers in its subsequent growth. Theoretically, the points of growth are the growing hyphal tips. If we assume that there are n such units per millimeter of the perimeter of the inner disk, the area of each "sector" becomes $S = (\pi R^2 - \pi r^2)/2\pi nr$, which, in turn, gives the following ratio $S_0 = \dfrac{(\pi R^2 - \pi r^2)}{2\pi nr} \times \dfrac{2\pi na}{(\pi A^2 - \pi a^2)}$, or $S_0 = \dfrac{a(R^2 - r^2)}{r(A^2 - a^2)}$, or in terms of the above $S_0 = (a/r)\ (DA_0)$.

Figure 2 diagrams that growth which is actually compared by each method of calculation. In the ring-area method, the shaded area in figure 2-A is compared for the control and the test mats. In the radial method the comparisons are based on the relative lengths of line X for the controls and the tests (*cf.* fig. 2-B). While the new method, the sector-area method, compares the respective areas as portrayed in figure 2-C.

[1] All letters with subzeros refer to ratios; *e.g.*, R_0 is the ratio of the radii, DA_0 is the ratio of the differences of the areas, etc.

It is a well known fact that the actual growth of a fungous colony takes place in all three dimensions; the order of their importance being *Radial* > *Tangential* > *Vertical*.

Those who argue in favor of the ratio in increases of radii (DR_0) maintain that radial growth predominates over the others to such an extent that measurements based on this one dimension of growth approximates the true ratio between the actual growths in all three dimensions. They further maintain that the ring-area (DA_0) method does not take into consideration the *amount* of fungus contributing to the next increment of growth. Since the radii ratio method is based entirely on linear growth, the amount of con-

FIG. 2. Diagrams illustrating different methods of comparing fungal growth. Explanation in text.

tributing material plays no part in subsequent calculations. The adoption of the ring-area method assumes that a comparison of a three dimensional growth is approximated more closely by taking into consideration the two more important dimensions than if the primary one (radial) alone is used. The exponents of this method object to the fact that those accepting the other method consider that all growth is linear with none occurring in the other planes. This objection is overrated since after the colonies have attained relatively large diameters, the growth does approach a linear function and growth in the tangential direction is far less important than the ring-area formula would lead one to conclude.

Since the radial method does not, in any case, take into consideration the growth in the second measurable dimension, and since the ring-area method fails to take into account the amount of fungous material present which contributes to this new increment of growth, a ratio-formula was devised in an attempt to overcome both of these objections and yet to incorporate the desirable features of the two older methods. This suggested the sector-area (S_0). The number of unit growing points does not necessarily mean the number of hyphal threads per millimeter; if that were true, this method would be nearly ideal and no objection of importance could be raised against its validity. It is to be remembered, however, that no ratio based on only one or two dimensions can be more than an approximation. Any formula, therefore, which gives a result closer to the actual condition (theoretical) is a far better approximation.

Several obvious, interesting, and important relationships can be seen. If the initial growths are identical, the value of S_0 and that of DA_0 are identical. If the conditions arise where the ring-area ratio becomes one, then $S_0 = a/r$, which is the reciprocal of R_0. These and other relationships can be most easily seen by actual calculations based on hypothetical data (*cf.* the table). All types of measurements given in the table were experienced in actual experiments conducted in the laboratory.

TABLE I

CALCULATIONS BASED ON HYPOTHETICAL DATA

CASE	a	A	r	R	DR_0	DA_0	S_0
I	1	2	1	2.5	1.50	1.75	1.75
II	5	7	7	9	1.00	1.33	0.95
III	2	3	3	4	1.00	1.40	0.93
IV	9	11	11	13	1.00	1.20	0.98
V	4	5	3	4	1.00	0.78	0.97
VI	6	8	3	5	1.00	0.57	1.14
VII	1	4	7	8	0.33	1.00	0.14
VIII	2	14	4	20	1.33	2.00	1.00
IX	6	9	4	6	0.67	0.44	0.67
X	3	9	10	15	0.83	1.74	0.52

Case I shows that the ring-area method gives the same value as the sector-area method when the original radii are identical. Under no other set of conditions is this possible. The only situation under which all three will yield the same factor is when $a = r$ and $A = R$. It is to be noted, however, that these two sets of conditioned measurements are exceptions rather than rules.

In cases II, III, IV, V and VI no effect is shown by the radial method, while either augmentation or inhibition is made evident by either of the other two methods of calculation. The ring-area method shows no effect for the measurements in case VII, while either of the other two types of calculation yield a factor of marked inhibition. Case IX is a special situation under which all three methods suggest marked inhibition. And in case X there is revealed an overemphasis on the tangential dimension by the ring-area method.

If we assume for the present that the sector-area method is the most accurate of the three, we may see the following relationships made evident by the table. (1) If the value of S_0 is 1.00, the other two methods show significant and marked augmentation (VIII). (2) If S_0 gives values showing inhibition, the value of DR_0 may show no effect (II, III, IV or V) or inhibition (VII, IX or X), while the DA_0 factor may suggest augmentation (II, III, IV or X) or no effect (VII) or inhibition (IX). (3) On the other hand, if the factor S_0 indicates augmentation, the DR_0 factor shows augmentation

to a lesser degree (I) or no effect (VI), while the DA_0 factor shows the same effect (I) or marked inhibition (VI). Other variations, although related to those above, may be shown by choosing another set of data.

It can be seen, therefore, that the sector-area method takes into consideration (1) growth in the radial direction; (2) growth in the tangential direcion; (3) the number of units contributing to the subsequent growth; (4) the relationships of initial and of total growths, and consequently, of the new increments of growth for the two colonies compared; and (5) the relative importance of radial and tangential growth quantities depending on previous conditions. The radial method fails to consider steps 2, 4, and 5 above. The ring-area method omits steps 3 and 4, and either exaggerates or diminishes markedly step 5.

It is to be remembered that the sector-area method is to be employed only for detecting the effect of a given substrate, as compared to a control, from one time interval to the next and for calculating successive growth rates of a given colony. The total-area method is best adapted for calculating the cumulative effects, while the total-radii, the difference of radii, and the ring-area methods prove to be inadequate for any type of measurement yet made for the growth of a fungal mat on solid nutrient media.

My sincere thanks are extended to Dr. B. M. DUGGAR, of the University of Wisconsin, for his critical review of the protocol.

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THE CALIBRATION OF A CONSTANT RECORDING ILLUMINOMETER WHEN THE SENSITIVE SAMPLING SURFACE IS HORIZONTAL[1]

DONALD R. WILLARD AND JOHN B. SMITH

(WITH TWO FIGURES)

Introduction

A recent issue of PLANT PHYSIOLOGY contained a description of a constant recording potentiometer developed by WALLACE (**3**), who subsequently suggested its use in conjunction with a Weston Photronic cell for recording sunlight for routine weather records (**4**). Arrangement and use of the apparatus was discussed in considerable detail, but no thorough attempt at calibration in terms of customary light units was reported. By arrangement with Dr. WALLACE, the instrument was purchased, and has been in intermittent use in this laboratory for considerable periods of time. The object of this brief paper is to report an attempt at calibration of the apparatus in foot-candles by comparison with results obtained with a Macbeth illuminometer and a Weston illuminometer.

This empirical comparison indicates the accuracy obtained under the specific conditions described. As described here, the instrument does not provide a complete record of light energy or its effects on plant functions. It does, however, offer possibilities of data for weather records with an accuracy greater than that from mere visual observation or from intermittent readings with light meters.

Procedure

The recording potentiometer was set with the zero point about eight millimeters from one edge of the paper, at a sensitivity such that the maximum sunlight caused the recorder pen to move about seven inches. It was activated by a Weston Photronic cell, with a ten-ohm shunt across the input terminals of the recorder. This shunt is necessary to secure an approximately linear relationship between foot-candles falling upon the cell and current output over the wide range of light incidence, since, according to the Weston Electrical Instrument Corporation (**2**) the current output of these cells is much more characteristic of the light intensity than is the voltage generated.

The cell was mounted horizontally on a suitable rubber base, upon a wooden platform built over an unused chimney on the roof of the laboratory. It was covered by an inverted, heavy, white glass, flat-bottomed bowl, which

[1] Published by permission of the Director of Research as Contribution no. 529 of the Rhode Island Agricultural Experiment Station.

rested upon a thick rubber disk. This bowl protected the cell from the weather, absorbed or reflected 75 per cent. of the light falling upon it, and was just large enough to cover the cell. The arrangement of the cell and bowl is shown in figure 1. The bowl was held down level on the rubber disk by suitable clamps, and the interior was connected to a small rubber balloon on the under side of the platform over the chimney. This provided for expansion and contraction of the air under the bowl, as suggested by Prof. C. I. GUNNESS, of Massachusetts State College. A few pieces of anhydrous calcium chloride were placed under the bowl to maintain low humidity.

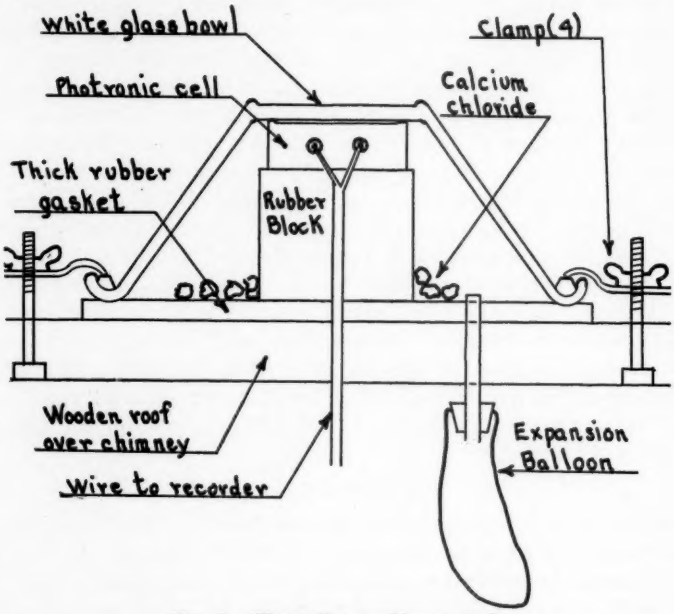

FIG. 1. Photocell assembly on roof.

The calibration was accomplished by means of foot-candle readings taken simultaneously with a Leeds & Northrup Macbeth illuminometer, equipped with a daylight absorbing screen, and a Weston illuminometer from horizontal reflecting surfaces, at stations within fifty feet from the photoelectric cell. The target of the Weston illuminometer was placed in a horizontal position at the same light station. These readings were plotted against the rise of the pen on the drum of the recorder in millimeters. The observations were made at the same time as the foot-candle readings. Actually about two minutes were required for observations, and care was exercised that the light intensity did not change appreciably during this time. Only clear days when the sky was free from clouds were selected.

Results

Foot-candle readings from both illuminometers were plotted against millimeters rise of pen on drum (fig. 2). This graph shows the agreement which may be expected between the Weston and Macbeth illuminometers. It will be noted that the two instruments agree quite well above 4000 foot-candles, but that below this value the curves separate, and apparently the Weston readings are too low. This may be attributed to the large angle of incidence, measured from the normal, when the sun is low on the horizon, causing reflection from the glass surface over the cell of the Weston illuminometer, and also to the shadow cast by the rim of the case holding the cell. Neither of these factors affects the readings of the Macbeth instrument.

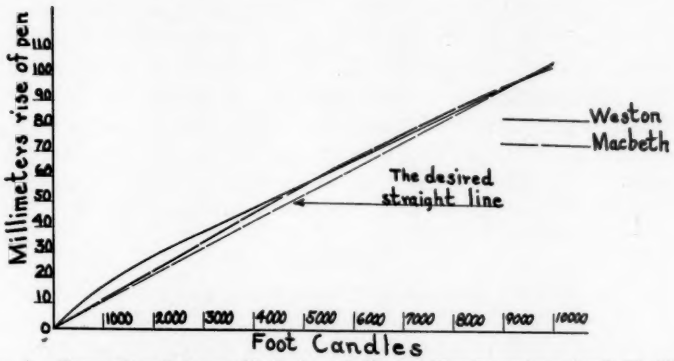

FIG. 2. Comparison between Macbeth and Weston illuminometers, April 30, 1937.

The graph also shows the desired straight line relationship between foot-candles falling on the cell, rise of pen in millimeters from maximum to minimum readings, and the divergence of the actual observations from this desired straight line. The reading of the pen is a little high throughout the entire range, the greatest divergence being at about 4800 foot-candles. This indicates that the relationship between rise of pen, or cell output, and foot-candles is not exactly a straight line. The maximum error is at 4800 foot-candles, where it is approximately 350 foot-candles or 7.3 per cent. The error decreases on both sides of this value.

Discussion

The resistance across the input terminals of the recorder cannot be reduced much below ten ohms and still have enough potential drop to drive sufficient current through the coil of the galvanometer to move it. Reducing this resistance also reduces sensitivity. The problem becomes one of how much sensitivity can be sacrificed in order to secure as nearly as possible a straight line relationship.

This relationship is important, because it makes possible measurement of the total light upon a surface over a period of time, by integrating the area under the curve drawn by the pen, and expressing the result as foot-candle hours, or in some other convenient unit. A ten-ohm shunt with the thick, white glass, flat-bottomed bowl inverted over the cell, gave better results than several other types of translucent shades that were tried.

Because of changes in the photronic cells, the calibration has not proved permanent, but must be redetermined frequently. Certain of the cells seem much more durable than others. Reduction of sensitivity of a cell may be classed as permanent or temporary. Apparently the cells may be permanently damaged by exposure to the high temperatures of bright summer days. The Weston Electrical Instrument Corporation state that 122° F. (50° C.) is the maximum endurable temperature, beyond which decided changes in sensitivity may be expected. They state also that moisture has a very harmful effect upon the cell (**2**). These two factors must be guarded against in the assembly of the sampling unit. A temporary failure may be remedied by unscrewing the backs of the cells and inserting small springs, as was done by WALLACE (**4**) who thoroughly discusses the troublesome weaknesses of the cells. Despite all precautions, however, changes in the relationships between light and the recorded data have occurred.

Conclusions

With a suitable cell mounted horizontally, and covered with a flat, white glass surface capable of removing 75 per cent. of the total light, the recording illuminometer may be calibrated for light falling on a horizontal surface, using either a Weston or a Macbeth illuminometer as a standard.

The maximum error should not be greatly in excess of 7 per cent. The error means variation of observations from the desired straight line relationship for any cause whatever, and does not consider errors in the Weston or Macbeth instruments. The calibration is not permanent, and must be repeated at intervals.

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MOLYBDENUM AS AN ESSENTIAL ELEMENT FOR HIGHER PLANTS

D. I. Arnon and P. R. Stout

(WITH ONE FIGURE)

The preliminary findings that molybdenum, in minute amounts, improved the growth of barley plants in a culture solution supplied with ammonium salts as the sole source of nitrogen (**1**), and that a group of seven heavy metals including molybdenum increased the growth of lettuce and asparagus (**2**), supported the view that the list of essential elements for higher plants is incomplete and suggested the desirability of determining whether or not molybdenum is essential in the nutrition of higher plants.

The essential nature of molybdenum for higher plants was established according to the criteria set up for other elements required by higher plants in minute quantity (**3**) as follows:

(A) When tomato plants were grown from the seedling stage in rigidly purified nutrient solutions (**13**) containing all of the eleven nutrient elements now regarded as essential (N, P, K, Ca, Mg, S, Fe, B, Mn, Zn, and Cu) characteristic deficiency symptoms became apparent in a few weeks. The lower leaves developed a distinct mottling different from any other deficiency symptom yet observed in the tomato (fig. 1A). In later stages necrosis at the margins and a characteristic involution of the laminae was evidenced (fig. 1 B). Almost all of the blossoms abscissed without setting fruit. These deficiency symptoms were produced in six successive experiments at various seasons extending over a period of a year and a half, using both purified and unpurified nutrient solutions; the results, however, were consistent only if the nutrient medium was rigidly purified by procedures efficacious in removing minute metal contaminants (**13**). Ordinary distilled water and C. P. chemicals contained molybdenum as a contaminant in amounts, adequate at times, to supply plant needs.

(B) The development of these deficiency symptoms was prevented by adding 1 part of molybdenum as molybdic acid to 100,000,000 parts of nutrient solution. This was effective whether added singly or jointly with 19 other elements,[1] none of which were capable of replacing molybdenum [The supplementary microelements' solutions B7 and C13 (**2**)].

(C) To test the direct effect of molybdenum on the plant as distinguished from its possible effect on the root environment, molybdenum-deficient plants were sprayed with a dilute solution of molybdic acid (0.05 p.p.m. Mo) so as to bring about absorption only through the aerial parts of the plant. Re-

[1] Ti, V, Cr, W, Co, Ni, Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, Be.

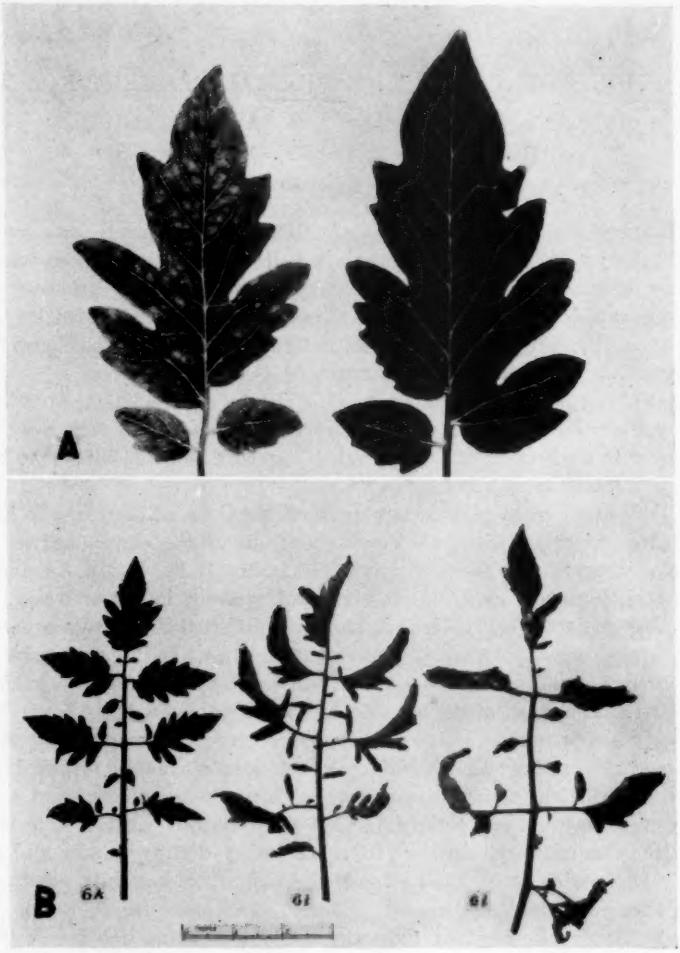

FIG. 1. Molybdenum deficiency symptoms.
A. Left, mottled appearance of foliage of a molybdenum-deficient plant; right, normal leaflet.
B. The two leaves on the right show curling and necrosis at the edges characteristic of an advanced stage of molybdenum deficiency; normal leaf at the left.

covery and resumption of normal growth with the disappearance of the molybdenum-deficiency symptoms took place.

Although but 0.01 p.p.m. was required to supply the needs of young tomato plants they tolerated relatively large concentrations of molybdenum

in the nutrient solution, as distinct injury from excess was obtained only if concentrations exceeded 10 p.p.m. (2 mg. of molybdenum per plant).

In recent years the biological importance of molybdenum, particularly with reference to the nutrition of lower plant forms, has been recognized. STEINBERG (**11**) found that molybdenum is essential for the growth and sporulation of *Aspergillus niger* and that it has a relation, in that organism, to nitrogen metabolism (**12**). The relation of molybdenum to nitrogen fixation and growth of Azotobacter has recently received considerable attention (**4, 6, 9**). As for higher plants, the widespread distribution of molybdenum has been noted (**8, 10**), and instances of improved growth as well as toxicity from adding molybdenum to the culture medium, have been reported (**5, 14, 7**).

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BRIEF PAPERS

SINTERED PYREX GLASS AERATORS

GEORGE H. CARROLL

(WITH ONE FIGURE)

Sintered glass aerators have been found to be of great value in water culture experiments. A few years ago PLANT PHYSIOLOGY carried a paper on sintered pyrex glass aerators.[1] This paper, however, did not treat of the actual steps involved in the making of the aerators. The present paper describes a technique for the rapid and economical production of such aerators. A sintered glass aerator is essentially a baked disk of fine glass particles, having sufficient porosity to allow the passage of fine air bubbles; this disk when sealed into a tube constitutes a complete aerator.

The first requirement in the production of an aerator is a quantity of pyrex glass particles of minute size and uniformity, free from impurities. A porcelain ball mill with quartz pebbles will reduce pyrex glass scraps to a fine size when rotated rapidly for a few hours. Particles of uniform size may then be obtained by means of an elutriator, as used by FURNSTAL and JOHNSON,[1] or by sifting through a wire screen, or a piece of silk bolting cloth. By the use of two pieces of cloth, one of 125 mesh per inch and one of 173 mesh per inch, a suitable size may be obtained. By rejecting all particles that do not pass through the larger mesh, and all that pass through the smaller mesh, an appropriate range is obtained. After washing with distilled water and drying, the ground glass is then poured into nickel rings (solid nickel) set on a plate of pure nickel, and the whole baked in a muffle furnace at a temperature of about 780° C. for about 20 minutes. If the furnace is not provided with a thermometer, the temperature may be estimated by observing the color of the heated interior; after a little experience one may safely trust this empirical method. By using a nickel ring cut from tubing of relatively small diameter, 14 mm., a greater number of disks may be accommodated at one time.

There are two ways of assembling the component parts of the aerator, one method requiring special skill in the art of glass blowing; the other, requiring no special skill, yields a product of less presentable appearance, but equally as good in point of service.

In the first method, a short piece of tubing is selected, an 8- to 10-cm. length with an inside diameter about 1 mm. less than the diameter of the sintered disk which is to be sealed in. This difference in diameter is important in facilitating rapid production in the disk-sealing phase. To each end

[1] FURNSTAL, A. H., and JOHNSON, BURKETT. Sintered pyrex glass aerators. Plant Physiol. 11: 189–194. 1936.

of the tube is sealed a length of small diameter tubing (see fig. 1, A). This composite piece is then cut exactly in the middle giving two identical pieces. The larger ends of these are then flared slightly to admit a disk. In the process of sintering, a disk acquires a slightly beveled edge, allowing it to fit snugly into the flared end of the tube. The small end of the tube is then passed through a funnel-shaped opening through a block of carbon. A brush from an electric motor (8 cm. × 5 cm. × 2 cm.) will do. When the end of the

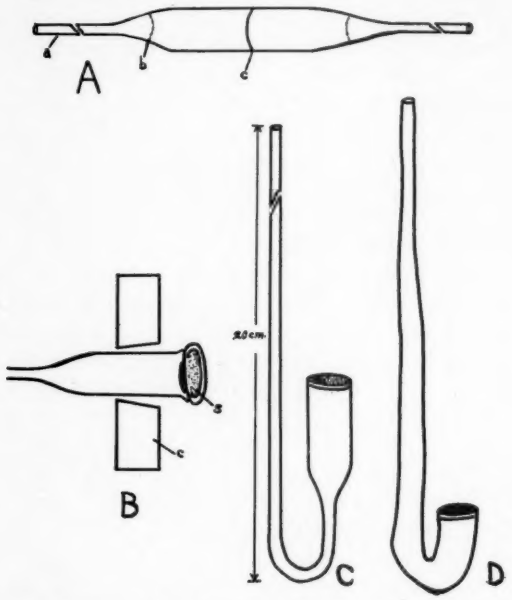

FIG. 1. A. Glass tubing sealed for preparation of two aerators: a, 20 cm. length of 8 mm. tubing; b, junction of 8 mm. and 14 mm. tubing; c, midpoint where cut is made after long pieces are sealed on each end. B. Cross section through carbon block: c, carbon block; s, sintered disk. C. The complete aerator. D. Hand drawn aerator.

tube holding the disk is heated soft, the tube may be drawn through the block and the disk will be firmly sealed in with one operation (see fig. 1, B). The carbon block must, of course, press the soft hot glass against the sintered disk. The junction of the disk and tube must then be annealed. The size of the smaller opening of the funnel-shaped hole through the carbon block must be as great as the diameter of the disk plus the thickness of the tube. An opening of smaller size will cause too great compression and tend to produce an impervious disk. The final step is the bending of the tube to bring the surface of the disk into proper position (see fig. 1, C).

The second method is much simpler than the preceding and is adapted to the tyro glass blower. In this method a single long tube is used, of an inside diameter slightly less (about 1 mm.) than that of the disk. After the disk is sealed in, as in the first method, the tube is heated and drawn out and bent as illustrated in figure 1, D. For this type, a pound of 14-mm. (outside diameter) pyrex tubing when cut to 20 cm. lengths will make about 24 aerators.

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NITROGEN NUTRITION IN RELATION TO YIELD AND QUALITY OF GRAPEFRUIT

WILLIAM E. MARTIN

Investigations at this and other stations have shown generally that nitrogenous fertilizers exert considerable influence in increasing the yields of grapefruit and other citrus fruits. The factors affecting quality of grapefruit and the relation of nitrogen nutrition to quality have not yet been fully studied with the result that orchard management practices, particularly in Arizona, cannot be made with the assurance of modifying both yield and quality.

A study of the physiology of fruiting of Marsh Seedless grapefruit was begun at this station in the autumn of 1937. Some results have been obtained which may suggest methods of rationalizing the cultural program in terms of treatments affecting both the yield and the commercial quality of the fruit produced.

These experiments indicate that a relatively high nitrogen content of the tissue at the time of blossoming is followed by a relatively large set of fruit. Present evidence suggests that high quality fruit at harvest follows a relatively low nitrogen content of the tree during the summer and fall and may be materially affected by cultural practices carried out during the growing season.

It is suggested that such practices as heavy nitrogen fertilization, with clean cultivation during the summer bring about a vegetative tree condition leading to the production of coarse-textured thick-skinned fruit largely of the lower commercial grades. On the other hand starvation practices such as the growing of competing cover crops, and the withholding of nitrogenous fertilizers during the summer months tend to bring about a low nitrogen composition of the tree, and a condition favorable to the production of smooth-textured, thin-skinned fruit of relatively superior market grades.

Data leading to this view were obtained by grading experimental lots of Marsh Seedless grapefruit at a commercial citrus packing house. The commercial grading of grapefruit is based to a considerable extent upon external appearance. Smooth, symmetrical, thin-skinned fruit are classified into superior grades, while rough, thick-skinned fruit fall into the lower commercial grades or are discarded as culls.

A summary of the experimental treatments, yields, and the proportion of fruit of each of the commercial grades is presented in table I. Yields have been calculated as percentages of the previous year's production.

The accompanying table shows that the trees in plots 1, 2, and 3 which had been given treatments to increase the nitrogen content of the tree prior

TABLE I

EFFECTS OF FERTILIZER AND CULTURAL PRACTICES UPON YIELD AND QUALITY OF MARSH SEEDLESS GRAPEFRUIT

PLOT	WINTER TREATMENT	YIELD	SUPPLEMENTARY TREATMENT		QUALITY AT HARVEST			
			SPRING	SUMMER	No. 1 SUNKIST	No. 2 CHOICE	No. 3 STANDARD	No. 4 CULLS
		%			%	%	%	%
1		196.8	High N	High N	13.7	27.8	34.5	24.0
2	High nitrogen fertilization	180.9	Starved	Starved	40.1	32.7	13.9	13.3
3		192.8	High N	Starved	32.4	31.4	19.4	16.8
4	Starvation practices	148.8	Starved	Starved	39.9	34.2	11.5	14.4
5		156.3	High N	High N	16.8	31.5	28.6	23.1
6		138.8	Starved	High N	19.4	26.3	26.5	27.8

to bloom, produced about 30 per cent. more fruit than did the trees in plots 4, 5, and 6 which were unfertilized during the winter and had been further starved by a competing cover crop of barley. The analysis of samples taken just before bloom showed much more nitrogen in the leaves and twigs taken from plots 1, 2, and 3 than was found in similar samples taken from plots 4, 5, and 6. Nitrogen fertilization during the winter as well as during the spring and summer was accomplished by the addition of calcium nitrate which is believed to be immediately available to the tree.

Summer treatments appear to exert a marked influence upon the commercial quality of the fruit at harvest. Plots 1, 5, and 6 received nitrogenous fertilizer during the summer and the trees became deep green in color, made flushes of summer growth, and produced a large proportion of culls and a small proportion of first grade fruit. On the other hand, plots 2, 3, and 4 which received no summer nitrogen applications and which were starved by the use of a competitive cover crop of Sudan grass, produced a relatively small proportion of culls and a large proportion of high quality fruit according to commercial grading. Fertilizer applications and starvation practices during the spring months appear to have had similar but lesser effects upon both yield and quality.

If present evidence is borne out by further studies, orchard management programs in Arizona could then be planned by considering the seasonal needs of the tree and nitrogen could be applied when it would do the most good in maintaining production, but be withheld or diverted by cover crops during the season of the year when its presence might impair the commercial quality of the fruit.

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NOTES

Annual Election.—The sixteenth annual election of the American Society of Plant Physiologists has resulted in the choice of Dr. JOHN W. SHIVE, New Jersey Agricultural Experiment Station, as president for 1939–1940. The other officers elected are Dr. F. P. CULLINAN, U. S. Department of Agriculture, vice-president; Dr. W. E. LOOMIS, Iowa State College, secretary-treasurer; Dr. D. R. HOAGLAND, University of California, member of the executive committee for three years; and Dr. B. E. LIVINGSTON, member of the board of editors for three years.

With these able leaders another year of progress and development is assured. As the Columbus meeting is approaching, the first duty of all members will be hearty cooperation with the new officers and the program committee in developing an adequate program. All arrangements should be made early to avoid the necessity of last minute decisions. Those who desire to appear upon the general programs should be ready to respond promptly to the call for papers. Arrangement of the papers in non-conflicting groups for parallel programs requires plenty of time for intelligent decisions.

Summer Meeting.—The Milwaukee and Madison meeting was the ninth summer meeting of the Society. The convention opened on Tuesday, June 20, with a symposium on photoperiodism at which Dr. A. E. MURNEEK presided. The discussion was opened by Dr. W. F. LOEHWING, who discussed the present general status of the problems of photoperiodic response. The hormone relations were discussed by Dr. K. C. HAMNER, and the anatomical and temperature modifications were stressed by Dr. R. H. ROBERTS. About 45 were in attendance at the opening session.

An informal luncheon for plant physiologists at the Hotel Schroeder was happily arranged by the program committee. On Tuesday afternoon the necessary business was transacted, following which several papers were read. These dealt with ethylene production and the climacteric in apples, carbon dioxide storage of strawberries and raspberries, and the influence of legumes on the nitrogen nutrition of other non-leguminous crops.

The interest shifted to Madison for meetings on Wednesday and Thursday, the University of Wisconsin being host to the general gathering of botanists. President DYKSTRA, and Dean FRED of the University extended welcome to their guests, and responses from the visiting groups expressed the pleasure of the guests at their reception. Wednesday afternoon two invitation papers were presented, one on the rôle of the more abundant mineral elements by G. F. THORNTON, and one on radiation as applied to some current physiological problems, by B. M. DUGGAR. Following these addresses, time was taken to visit Professor DUGGAR'S radiation laboratories.

The final meeting on Thursday morning was devoted to a group of miscellaneous papers of general interest. After lunch, inspection of the laboratories for synthetic nitrogen fixation, and the biochemistry laboratory was the chief interest of laboratory men. The field men were personally conducted to the agronomy field experimental plots. At 4:00 P.M. a joint picnic for all groups closed the meeting.

While the attendance was lighter than usual, those who did come felt well repaid, and the committe in charge of arrangements, Dr. W. E. TOTTINGHAM, Dr. I. L. BADWIN, and Dr. L. F. GRABER deserve praise, and sincere thanks for the able handling of the convention arrangements.

New England Section Meeting.—The New England Section held its sixth annual meeting at Yale University, May 12–13, 1939. In addition to the presentation of papers concerned with the results of investigations, there was a special meeting for those engaged in blueberry and cranberry research. The session devoted to teaching problems was well attended. It was voted to accept an invitation to meet at Dartmouth College in 1940, with Dr. C. J. LYON, chairman, Dr. R. H. WALLACE, vice chairman, and Dr. L. H. JONES, secretary-treasurer.

Editorial Service.—The changes in the editorial service ordered at the Indianapolis meeting in 1937 increased the necessary work and keeping of records very materially. With the doubling in size of our official journal, the editorial responsibilities had grown to such an extent that there was not enough time to take care of all of the required work in a single office. The handling of manuscripts previous to their use interfered with the progress of the journal through the processes of manufacture. Serious delays arose, and these have forced us to adopt certain changes to relieve the pressure upon the editorial functions. Every member of the Society should note these changes, and cooperate with us to render the service most promptly and efficiently.

The receipt and handling of papers during critical examination and revision has been placed in another office. Dr. WALTER F. LOEHWING, Department of Botany, State University of Iowa, Iowa City, Iowa, has consented to serve as secretary to the editorial board. All manuscripts should be sent directly to him. He will send them to readers in whose field the papers fall, communicate with the authors as to advisable revisions, and assume full responsibility for care of the manuscripts until they have been passed upon by the editorial readers. Papers which successfully pass these requirements are then transmitted to the editor-in-chief, presumably to be used, unless there are obvious reasons for some other course. The editorial office will concentrate upon the problems of getting the journal out on time, meticulous editing of the papers, indexing of volumes, and all other matters

concerned with the issuance of the journal, preparation of author reprints, etc. This division of labor, it is hoped, will render the service less burdensome, lead to more prompt and efficient handling of our publication activities, and yet maintain the same standards of excellence for PLANT PHYSIOLOGY. Authors of papers are requested to send their manuscripts directly to Dr. LOEHWING; and please do not ask us to read and advise about papers which are not yet ready to be submitted. There is no time available for such advisory services.

Chemical Methods Committee.—The committee on analytical methods is one of the oldest standing committees of the American Society of Plant Physiologists. It was constituted almost at the beginning of our organized work, and for nearly 14 years has served the need for accurate information on analytical methods. The first reports were published in the first volume of PLANT PHYSIOLOGY in 1926, and from time to time additional reports were published, supplemental to the original papers. The most recent contribution of the committee, as yet unpublished, describes the methods of analysis of the main mineral elements found in plant materials. This report is to appear in the October number of the official journal.

Dr. TOTTINGHAM gave unstinting and distinguished service as chairman of this committee throughout the period since the committee was first organized. In accordance with his expressed desire to be relieved of his duties in connection with the committee's work, president LOEHWING undertook a revision of the personnel. As now constituted, the committee chairmanship is held by Dr. W. E. LOOMIS, who has served on the committee since the beginning. Other members holding over are Dr. Z. I. KERTESZ, and Dr. T. G. PHILLIPS. The newly appointed members are Dr. J. T. SULLIVAN, who is physiologist in the regional laboratory for pasture research, U.S.D.A., at the Pennsylvania State College, and Dr. A. G. NORMAN, formerly in charge of the biochemical section at the Rothamsted Experimental Station, now professor of soils at Iowa State College. The committee has made a distinct contribution to physiological research, and will undoubtedly continue to render valuable service in developing better methods, or evaluating older procedures.

Algae.—Under the title *Algae, the Grass of Many Waters,* Dr. LEWIS H. TIFFANY, head of the Department of Botany at Northwestern University, presents an interesting and valuable story of fresh water and marine algae. A great deal of the information given in this book will be of interest to physiologists. There are 13 chapters, many of them brief, and the entire work comprises only 171 pages. The chapter titles sufficiently indicate the contents. They are as follows: what are algae? algae and the foods they make; how algae grow and reproduce; algae of lakes and ponds; algae of streams

and rivers; algae of the sea; algae of the soil; algae of ice and snow; algae of bizarre abodes; algae of the past; algae and human welfare; how to collect algae; and how to study algae. A list of general literature references, and an index conclude the volume. Written in delightfully informal style, it appeals very strongly to the student, should make many friends for the science of algology, and for the author. The book is published by Charles C. Thomas, Springfield, Illinois, who quotes the price as $3.50 per copy. There are more than 50 illustrations.

Annual Review of Biochemistry.—Volume VIII of the *Annual Review of Biochemistry* contains twenty-five excellent reviews, most of which are valuable to the plant physiologist. We have come to expect these reviews to be first class summaries of the fields included, and they more than fulfill the expectations. The first review covers the field of biological oxidations and reductions. The dehydrogenases, flavoproteins, mutases, polyphenol oxidases, uricase, dioxymaleic oxidase, catalase, ascorbacid, citric acid, etc., are given consideration, as well as the unsatisfactory nomenclature, and the problem of potentials. The author, MACOLM DIXON, is himself a contributor in this field; he has handled the review in masterly fashion.

The proteolytic enzymes are reviewed by K. LINDERSTRØM-LANG, and nonproteolytic enzymes by K. MYRBÄCK. A number of the reviews are devoted to metabolism, the chemistry and metabolism of the compounds of sulphur, by G. MEDES; carbohydrate metabolism, by I. L. CHAIKOFF and A. KAPLAN; lipid metabolism, by W. M. SPERRY; metabolism of proteins and amino acids, by R. W. JACKSON and J. P. CHANDLER; mineral metabolism: calcium, magnesium, and phosphorus, by D. M. GREENBERG; metabolism of brain and nerve, by J. H. QUASTEL. Of special value are the reviews on chemical aspects of photosynthesis, by H. GAFFRON; mineral nutrition of plants, by J. W. SHIVE and W. R. ROBBINS; growth hormones of the higher plants, by F. W. WENT; and the biochemistry of yeast, by E. I. FULMER. Space does not permit mention of all of the papers, but they are all conscientiously prepared, and carefully edited. From the beginning of the series the *Annual Review of Biochemistry* has maintained the highest standards of excellence. Volume VIII may be purchased for $5.00 from Annual Reviews, Inc., Stanford University, California.

Experiments in Plant Physiology.—A laboratory manual of plant physiology adapted to American laboratories has long been needed. An attempt to meet this need has been made in the publication of *Experiments in Plant Physiology* by the McGraw-Hill Book Co. The authors are W. E. LOOMIS, Iowa State College, and C. A. SHULL, the University of Chicago. The manual outlines 167 experiments, from which anyone may select experiments of

elementary or intermediate grade, and adapt the work to the needs of the students, or to the equipment of the laboratory, or to the time available for instruction, quarter, semester, or year. The price quoted by the publishers is $2.00 per copy.

Plant Physiology.—Another very ably written text has been added to those now available to students of plant physiology. Dr. B. S. MEYER and Dr. D. B. ANDERSON'S *Plant Physiology,* published by D. Van Nostrand Co., New York, belongs among the better works devoted to the physiology of plants. The chapters follow an orderly, logical sequence, and the student is prepared to understand the significance of processes by a thorough grounding in the principles of biophysics and colloidal chemistry. The authors are skilful teachers, and have deployed their material in admirable fashion. We hope that every student of plant physiology may read and thoroughly assimilate the information presented. It would probably be impossible to write a text so skilfully that there would not be some criticisms to offer, or differences of opinion as to interpretation. This work, however, is relatively free of flaws, and leaves little to be desired as a statement of current facts and theories in its field. It is a very commendable effort; its thirty-seven chapters are so many fine excursions into the realm of plant functions, and both students and teachers will appreciate the opportunity of using this excellent text. The price is $4.50 per copy.

Elements of Plant Pathology.—A welcome addition to the books on plant pathology is now available. *Elements of Plant Pathology,* by Dr. I. E. MELHUS and Dr. G. C. KENT, of Iowa State College, is an elementary work, suitable for beginners, and also a work that should appeal to laymen in botanical affairs. It is well written, and fully illustrated (259 figures). The first 7 chapters present the general principles of plant pathology, after which the diseases are treated in systematic fashion, following the classification of the organisms causing disease. The chapters deal with diseases caused by phycomycetes, bacteria, viruses, ascomycetes, fungi imperfecti, basidiomycetes, seed plants, nematodes, and non-parasitic agents. It forms an admirable introduction to the subject, and deserves an enthusiastic reception. The publishers, Macmillan Co., 60 Fifth Ave., New York, offer it to the public at $4.00 per copy.

Dictionary of Scientific Terms.—A third edition of HENDERSON and HENDERSON'S *Dictionary of Scientific Terms* has been prepared by J. H. KENNETH. Previous editions were published in 1920, and 1929. A casual examination indicates that more than ten thousand terms are included. There will always be differences of opinion about the adequacy of the definitions

given for the terms included. For instance, the word hormone is defined, for plants, as "internal secretions in plants, as wound or digestive secretions." Would hormone students finds the definition acceptable? And should general metabolites be referred to as internal secretions in this case? Such works, however, have a valuable place in the libraries of students and laymen alike. Frequently one encounters terms whose meanings are not suggested by the context, and nothing short of a dictionary suffices in such cases. It is very useful, therefore, to have such a volume handy on one's desk. The publishers are D. Van Nostrand Co., 250 Fourth Ave., New York. The quoted price is $7.00 per copy. It contains 383 pages, and is unillustrated. Terms are included for Botany, Zoology, Physiology, Anatomy, etc.